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CONTRIBUTION TO THE KNOWLEDGE OF *STACHYBOTRYS* AND *MEMNONIELLA* IN CULTURE

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ABSTRACT

Members of *Stachybotrys* and *Memnoniella* are morphologically and physiologically closely related and are of worldwide distribution. Many of them are commonly isolated from soil and are capable of utilizing cellulose and damaging fabrics made of plant fibers. Cultures of *Stachybotrys chartarum* and *Memnoniella echinata* have been used for fungus resistance tests in U. S. and British military specifications. Strains of *S. chartarum* are also known to produce toxic compounds which have been reported as agents of stachybotryotoxicosis in animals and man. The toxicants belong to the series of sesquiterpenoid mycotoxins classified as 12,13-epoxy-Δ^9-trichothecenes. The salient feature both genera have in common is the production of macronematous conidiophores with an apical cluster of several unicellular conidiogenous cells bearing unicellular enteroblastic-phialidic conidia. The main difference between *Stachybotrys* producing slimy heads of conidia and *Memnoniella* producing chains of conidia is in the length of time between formation of the septum in the phialide neck and its splitting when the conidia separate. In *Stachybotrys* the new conidia arise after previous ones are mature and released from the phialide neck, whereas in *Memnoniella* the

new conidia arise in basipetal succession before the previous ones are mature. In the present study fifty strains currently maintained in the American Type Culture Collection (ATCC) were characterized and reidentified to the following species: *Stachybotrys albipes*, *S. bisbyi*, *S. chartarum*, *S. cylindrospora*, *S. dichroa*, *S. kampalensis*, *S. microspora*, *S. nephrospora*, *S. oenanthes*, *S. parvispora*, *S. theobromae*, *Memnoniella echinata*, *M. subsimplex*. Each species treated has been redescribed and illustrated. A key is constructed to facilitate the identification of these cultured species. The shape, size and the color and ornamented surface of phialoconidia are principally used to distinguish the species. Two new combinations, *Stachybotrys albipes* (Berk. & Br.) Jong & Davis and *S. microspora* (Mathur & Sankha) Jong & Davis, are proposed. *Stachybotrys sacchari* (Srinivasan) Barron is recommended as a synonym of *S. bisbyi* (Srinivasan) Barron, and *S. reniformis* Tubaki and *S. sinuatophora* Matsushima are synonyms of *S. nephrospora* Hansford.

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INTRODUCTION

The Hyphomycetes, grouped together because of similarities in their morphological appearance, belong to a specially classified group known as the Fungi Imperfecti. The incomplete type of life cycle in the imperfect fungi is identical with the asexual states of the Ascomycetes or of the Basidiomycetes. Ideally, the scientific names of imperfect (asexual) fungi should be discarded and replaced by the names of their perfect (sexual) states according to the natural system. However, some of the imperfect species, recognized as playing important roles in agriculture, industry, medicine and environment, have never been detected in their perfect states. They are among the most common of aquatic and soil fungi. The Hyphomycetes especially are abundant everywhere, many of them being pathogenic to plants, animals and humans. Accordingly, Fungi Imperfecti cannot be disregarded, and they are grouped exclusively in the class Deuteromycetes, which has long been classified independently of the Ascomycetes and the Basidiomycetes.

The necessity of living cultures for characterizing, identifying, and classifying the Hyphomycetes has been emphasized in recent years by the application of new taxonomic criteria related to the nature of conidiogenous cells and the precise method by which conidia are produced (Hughes, 1953; Goos, 1956; Simmons, 1966; Barron, 1968; Tubaki, 1958; Kendrick, 1971; Kendrick & Carmichael, 1973). Although Barron (1968) and Kendrick and Carmichael (1973) have recently applied the new scheme of classification to nearly 600 genera of Hyphomycetes and Ellis (1971a) has provided modern descriptions and illustrations of the common dematiaceous Hyphomycetes, our present knowledge of the new taxonomic criteria still has not come close to satisfying our need in identification of unknown Hyphomycetes. Therefore, much confusion has resulted in the modern literature, when, at different times, mycologists have set up new genera and new species based on different sets of criteria, e.g., superficial or developmental characters, or combinations of both. It is also difficult to correlate the original descriptions in the classical literature with the new scheme because most of them are hopelessly inadequate and without any indication of the method of conidial production. For these reasons, difficulties in determining the identities of Hyphomycetes in culture are frequently encountered by us in our routine curatorial work at the American Type Culture Collection (ATCC).

The specific aims of the project are: (1) to study as many strains as possible of selected genera of Hyphomycetes which are presently available from reliable culture collections; (2) to determine the identity and morphological variability of each strain and to utilize the results as a basis for resolving the formidable nomenclatural problems associated with Hyphomycetes in culture and in nature; (3) to describe and illustrate the species examined in culture so as to evaluate the more detailed microscopic characters used as criteria in the new scheme of classification; and (4) to construct a key to facilitate the identification of cultured species on a genus by genus approach.

The descriptions, illustrations and keys which result from these studies will be extremely useful in identifying Hyphomycetes in culture, especially those isolated from such materials as soil, water, humans, animals and plants. The present report deals with the genera *Stachybotrys* and *Memnoniella*.

HISTORICAL TREATMENT OF THE GENERA

The imperfect genus *Stachybotrys* was proposed by Corda (1837) for a single species *S. atra* Corda. He described the genus as follows: "Stipes septus, ramosus; ramis apice ramulis verticillatis, mamillaribus, brevissimis, capitulum formantibus corematis; sporis didymis, homogenesis regulariter postis et capitulo innatis."

A detailed review of the earlier literature of the genus has been presented by Bisby (1943). From critical studies of cultures and herbarium specimens, he emended the genus as having the "Hyphae, phialophores, and phialides hyaline, brightly colored, or dark; strands or ropes of hyphae may be produced. Conidia (slime-spores) one-celled, normally dark and accumulating into a cluster." At that time there were over twenty species proposed in the literature, but Bisby reduced them to two distinct species, viz., *S. atra* and *S. subsimplex* Cooke. The type

species *S. atra* was later identified by Hughes (1958) with *Stilbospora chartarum* Ehrenberg ex Link. *Synsporium* Preuss was considered by Hughes as a synonym of *Stachybotrys*.

Fuckelina Saccardo (1875) was created to accommodate the conidial state of *Eriosphaeria raripila* Sacc. as *F. socia* Sacc. Saccardo (1878) later identified *E. raripila* with *Melanopsamma pomiformis* (Pers. ex Fr.) Sacc.; and Ferraris (1909) transferred *F. socia* to *Stachybotrys* as *S. socia* (Sacc.) Sacc.

Gliobotrys Höhnel (1902) was erected for a *Stachybotrys*-like species with hyaline conidiophores and conidia olivaceous in masses. The type species *G. alboviridis* Höhnel was later identified by Höhnel (1923) as the conidial state of *Melanopsamma pomiformis* (Booth, 1957). Therefore, *Fuckelina* and *Gliobotrys* must be synonyms and should be considered under *Stachybotrys*.

Hyalostachybotrys was established by Srinivasan (1958) to include two hyaline *Stachybotrys*-like fungi. Barron (1964) believed that a genus based on color of the conidia would have no taxonomic validity, and suggested that *Stachybotrys* should include not only the dematiaceous species but also the hyaline forms. He therefore reduced *Hyalostachybotrys* to synonymy with *Stachybotrys*.

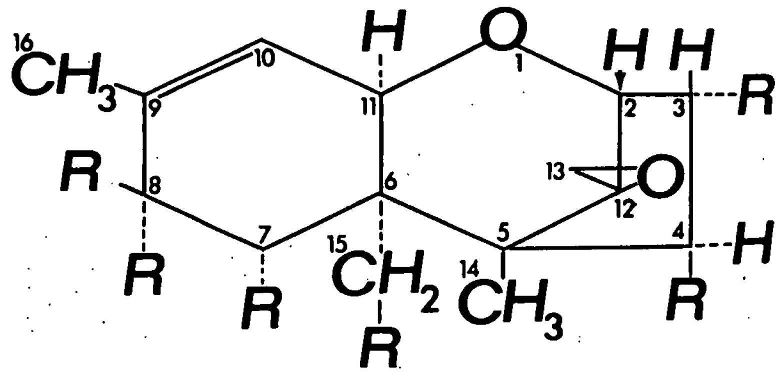
The genus *Memnoniella* was erected by Höhnel (1923) to include those fungi which have the conidiophores with an apical cluster of several swollen phialides resembling those of *Stachybotrys* and the black phialoconidia borne in long chains recalling those of *Aspergillus niger* van Tieghem (Galloway, 1933). Although Zuck (1949) found that some isolates of the type species *M. echinata* (Rivolta) Galloway might occasionally produce a *Stachybotrys*-like phase in culture much like the description of *S. subsimplex sensu* Bisby (1943), he considered *Memnoniella* and *Stachybotrys* to be distinct genera. The morphological distinction between these two genera is the disposition of conidia which are in long chains in *Memnoniella* and in slimy masses in *Stachybotrys*. Nevertheless, Smith (1962) considered this distinction not sufficient to warrant separation of the two genera and transferred *M. echinata* to *Stachybotrys*. Kendrick and Carmichael (1973) recently accepted Smith's taxonomic treatment, though the

arrangement of conidia was used by them as one of the four independent character sets to characterize and order the hyphomycetous genera for identification. On the other hand, Padwick (1945), Deighton (1960), Matsushima (1971a,b; 1975) and Ellis (1971a) recognized *Memnoniella* as being distinctive from *Stachybotrys*. The culture of the *Memnoniella* examined in the course of the present study remain stable in the characters distinguishable from *Stachybotrys*, therefore we also consider the two genera to be distinct taxa.

ECONOMIC SIGNIFICANCE

Members of *Stachybotrys* and *Memnoniella* are of worldwide distribution. Many of them are commonly found in soil and are capable of utilizing cellulose and damaging fabrics made of plant fibers. Cultures of the species *S. chartarum* and *M. echinata* have been used for fungus resistance tests in U. S. and British military specifications. Strains of *S. chartarum* (syn. *S. atra*, *S. alternans*) are also known to produce mycotoxins which have been reported as agents of stachybotryotoxicosis in animals and man (Gray, 1971; Rodricks & Eppley, 1974).

According to Forgacs (1965, 1972), stachybotryotoxicosis was first reported in horses from the Ukrainian S.S.R. in 1931. At that time it caused the death of thousands of horses in the Soviet Union. This disease has since been discovered to affect both animals and man, causing cutaneuous and mucosal necrosis. It also may bring about leukopenia and agranulocytosis, and may finally affect the heart, causing standstill in systole (Forgacs, 1972; Palyusik, 1970a). The *Stachybotrys* toxins as a group contain certain compounds in common, but they differ somewhat in their chemical structures and biological effects (Korpinen *et al.*, 1974). They belong to the series of sesquiterpenoid mycotoxins classified as 12,13-epoxy-Δ^9-trichothecenes (Eppley & Bailey, 1973). The following formula shows the structural features of these toxicants.



The toxic compounds can be obtained from oat cultures as well as from synthetic agar cultures of *S. chartarum* by extraction with diethyl ether in a Soxhlet apparatus (Forgacs & Carll, 1962; Palyusik, 1970a, Korpinen & Ylimaki, 1972; Eppley & Bailey, 1973). The degree of toxicity varies in different strains (Forgacs *et al.*, 1958; Palyusik, 1970a; Korpinen & Uoti, 1974). In addition to *S. chartarum*, the fungi which have been shown capable of elaborating 12,13-epoxy-Δ^9-trichothecenes include *Cephalosporium crotocinigenum, Fusarium concolor, F. equiseti, F. nivale, F. scirpi, F. tricinctum, Gibberella zeae, Myrothecium roridum, M. verrucaria, Trichoderma viride,* and *Trichothecium roseum* (Bamburg & Strong, 1971; Wilson, 1973). These naturally occurring members of the trichothecenes are colorless, crytalline, optically-active solids with low water solubility. They are chemically quite stable when stored under laboratory conditions and are not destroyed by usual cooking procedures (Bamburg & Strong, 1971). A toxic substance extracted from a strain of *S. chartarum* was found to be cytotoxic even at lower concentrations than aflatoxin B_1 (Bodon & Palyusik, 1970).

Feed contaminated with *S. chartarum* is the main source of the agent causing stachybotryotoxicosis in animals. Under field conditions, toxicosis can affect horses, cattle, hens, hippopotamus, bison, sheep, buffaloes, swine and poultry (Palyusik, 1970a; Rodricks & Eppley, 1974). Stachybotryotoxicosis may occur in man exposed to the fungus (Drobotko, 1945). The toxins are said to be absorbed through the skin and by inhalation, and illness may follow exposure from the handling or burning of hay molded by the fungus (Emmons *et al.*, 1970; Rodricks & Eppley, 1974).

PHYSIOLOGICAL CHARACTERS

Marsh and Bollenbacher (1946) showed that *S. chartarum* (as *S. atra*) and *M. echinata* were similar in many physiological characteristics, including the ability to grow on synthetic medium containing only biotin, glucose and inorganic salts. Perlman (1948) and Jermyn (1953) further confirmed their conclusions, and found that both fungi can utilize some twenty sugars and sugar derivatives as sole sources of carbon for growth. Growth was increased with the addition of traces of iron, zinc or manganese to the medium but was decreased with the addition of nickel, cobalt, chromium or aluminum.

Biotin deficiency was reported to be characteristic of *M. echinata* and *S. chartarum* (Marsh & Bollenbacher, 1946; Perlman, 1948). The absolute requirement of biotin was usually less than 5 µg per liter. However, the presence of aspartic acid in the culture medium was shown to reduce the amount of biotin required (Perlman, 1948). Lilly and Barnett (1951) noted that the variability of cellulolytic activity in strains of *M. echinata* in laboratory tests was possible correlated with biotin deficiency. The relationship between the amounts of sugar and biotin necessary for sporulation was described by Buston and Basu (1948). Less biotin was required for vegetative growth than for sporulation. Sporulation was conditioned by the concentrations of biotin and of the sugars present.

Perlman (1951) reported that *M. echinata* required biotin for spore germination. Growth from a spore inoculum required biotin, which was not replaceable by

desthiobiotin. However, if desthiobiotin was added to a growing culture supplied with limiting amounts of biotin, the desthiobiotin elicited a significant growth response. Since the mycelium is able to convert desthiobiotin to biotin but the spore cannot, the spore germination is affected by growth factor deficiencies (Cochrane, 1958).

Cultural studies done by Barron (1962) also indicated that *S. bisbyi* (as *S. aurantia*) had a rapid growth rate and, on a minimal synthetic medium, required a biotin supplement for normal growth, as reported in both *S. chartarum* and *M. echinata*.

Stachybotrys chartarum was found to synthesize humic acid-type polymers (Martin & Haider, 1969). The amino acid composition of *S. chartarum* humic acid was reported to be similar to that of soil humic acids (Ortiz de Serra *et al.*, 1973). Additions of a small amount of clay minerals, especially montmorillonite, to well-aerated cultures of this fungus greatly accelerated growth, glucose utilization, CO_2 evolution, phenol synthesis, and phenolic polymer formation (Filip *et al.*, 1972a,b). Nitrate-N was a poor nitrogen source for *S. chartarum*, but was a relatively good source of N in the presence of montmorillonite (Bordietti *et al.*, 1971).

Memnoniella echinata was reported to form acetic acid from carbohydrates (Perlman, 1951).

In tests of the microbiological deterioration of cellulosic fibers, a few strains of *S. chartarum* and *M. echinata* were found to be definitely active (Thom *et al.*, 1934; Greathous *et al.*, 1942; White *et al.*, 1948, 1949; Marsh *et al.*, 1949; Reese *et al.*, 1950). *Stachybotrys chartarum* synthesizes at least three enzymes capable of hydrolyzing β-glucosidase (Jermyn, 1955a), cellobiase (Youatt, 1958), and cellulase (Thomas, 1956). The specificity of these enzymes and their relationship was summarized by Youatt and Jermyn (1959). *Stachybotrys chartarum* produces the β-glucosidase over a wide range of culture conditions. The induction and biochemistry of this β-glucosidase has been intensively studied by Jermyn (1955a,b,c; 1962; 1965a,b,c; 1966a,b). Thomas (1956) reported that the optimum pH for the crude cellulase preparation from *S. chartarum* was 6.5-8.0.

Cochrane (1958) noted that pectin-polygalacturonase was found to occur in *S. chartarum*.

Of the 27 genera including 52 species of fungi, 6 species of Actinomycetes and 5 genera including 10 species of bacteria tested, Butt and Ghaffer (1972) found that *S. chartarum* (as *S. atra*) was able to inhibit the growth of 95.7% of the fungi, all the Actinomycetes and 83.3% of the bacteria.

MORPHOLOGY AND DEVELOPMENT

Members of *Stachybotrys* and *Memnoniella* are morphologically and developmentally closely related (Zuck, 1946; White *et al.*, 1949; Campbell, 1975). The unifying feature in common is the production of macronematous conidiophores with an apical cluster of several unicellular conidiogenous cells bearing unicellular enteroblastic-phialidic conidia (Barron, 1968; Kendrick & Charmichael, 1973). The genera differ primarily in the arrangement of the conidia, which are aggregated in slimy heads in *Stachybotrys* and in long persistent chains with the youngest conidium at the basal end of the chain in *Memnoniella* (Ellis, 1971a).

The conidiogenous cells of *Stachybotrys* and *Memnoniella* are considered to be phialides (Hughes, 1953; Tubaki, 1958; Barron, 1968; Ellis, 1971a; Kendrick & Carmichael, 1973). The phialide was defined by Kendrick (1971) as a conidiogenous cells in which at least the first conidium initial is produced within an apical extension of the cell and the conidium is literated by the rupture of the upper wall. Thereafter, from a fixed conidiogenous locus, the second and subsequent conidia are developed, each clad in an entirely new wall which is not derived from any existing layers of the wall of the conidiogenous cell. Any phialide wall distal to the conidiogenous locus is the collarette. The length of the phialide does not change while conidia are being produced.

However, the electron microscopical studies of phialoconidiogenesis in *Aspergillus clavatus* (Hanlin, 1976), *A. flavus* (Bojović-Cvetić & Vuyičić, 1974), *A. fumigatus* (Ghiorse & Edwards, 1973), *A. giganteus* (Trinci *et al.*, 1968), *A. nidulans* (Oliver, 1972), *A. niger*

(Tsukahara, 1970), *Neurospora crassa* (Lowrey *et al.*, 1967), *Metarrhizium anisopliae* (Hammill, 1972), *Verticillium albo-atrum* and *V. nigrescens* (Buckeley *et al.*, 1969), *Penicillium claviforme* (Zachariah & Fitz-James, 1967; Fletcher, 1971), *P. clavigerum* and *P. corymbiferum* (Fletcher, 1971), *Phialocephala dimorphospora* (Carroll & Carroll, 1974), *Phialophora richardsiae* (Olah & Reisinger, 1974) *Stilbothamnium nudipes* (Roguebert & Abadie, 1973), and *Trichoderma saturnisporum* (Hammill, 1974) have further refined the concept of phialoconidium formation. The characteristic features of phialoconidiogenesis are: (1) the inner cell wall of the conidiogenous cell gives rise to the conidium wall; (2) the successive production of conidia from a fixed conidiogenous locus inside the phialide shows no elongation once its tip is ruptured; (3) the phialoconidia are determined by transverse septa; and (4) the conidia are produced individually as discrete units to form slimy heads, or successive conidia are connected by a common septum to form persistent chains.

Campbell (1972; 1975) published the ultrastructure of conidiogenesis in *Stachybotrys chartarum* (as *S. atra*) and *Memnoniella echinata*. His interpretation and electron micrographs of both fungi appear essentially in agreement with those described for other enteroblastic-phialidic fungi mentioned above.

In *Stachybotrys* conidiophores arise from either intercalary or terminal cells of the mycelium. They are macronematous, morphologically different from purely vegetative hyphae, and determinate, i.e., the growth in length of the conidiophores ceases with the production of the first phialide. As the first phialide enlarges into its typical shape a cross septum is formed. Thereafter other phialides develop successively in a verticillate fashion below this septum, so that the first-formed phialide usually becomes the central one of the group. The wall of the phialide is continuous with the wall of the conidiophore. According to Campbell (1972) the septa which delimit the phialides from the conidiophore have a single central pore.

Phialides are always unicellular, generally cylindrical and usually slightly swollen in the upper portion before they taper to a narrow conidiogenous neck. The forming conidium first appears as a small bulbous

enlargement at the tip of the phialide neck. Since the outer layers of the phialide wall break very soon after the first conidium starts expanding, the opening of the phialide neck becomes the collarette; this is very short, but distinctive even under the light microscope. The growth in length of a phialide ceases with the rupture of the phialide neck. All conidia appear to develop successively in a fixed conidiogenous locus just beneath the opening in a phialide. The wall of the conidia originates from the inner layer of the phialide tip. The succession of conidial formation causes the inward thickening of the inner wall layers of the collarette (Campbell, 1972).

Phialoconidia are always unicellular and acrogenous. The contents of the conidiogenous cell pass through the opening and the conidia take shape immediately on the outside of the collarette. They are enteroblastic in that the outer layers of the phialide wall are not involved in the formation of the conidial wall (Kendrick, 1971; Ellis, 1971). Just prior to dehiscence, a cross-wall is laid down at the base of the mature conidium and at this point the conidium is released from the phialide (Campbell, 1972). Conidia are produced singly and successively as separate units and produce a slime that forms a mucilaginous mass which envelops the tips of the phialides.

As mentioned earlier, *Memnoniella* and *Stachybotrys* differ morphologically in the arrangement of the conidia. Developmentally they are similar, except that the phialoconidia in *Memnoniella* are produced in a basipetal succession and are held to each other by a common septum in long persistent chains. According to Campbell (1975), the main difference between *Memnoniella* which produces chains of conidia and *Stachybotrys* which produces slimy heads is in the length of time between the formation of the septum in the phialide neck and its splitting when the conidia separate. In *Stachybotrys* the new conidia arise after previous ones are mature and have been released from the phialide neck, whereas in *Memnoniella* the new conidia arise in basipetal succession before the previous ones are mature. Essentially the same pattern of development of conidial chains seen in *Memnoniella* is also found in several other imperfect genera, e.g., *Aspergillus*, *Paecilomyces* and *Phialomyces* (Subramanian, 1972).

The shape, size, color, and ornamented surface of phialoconidia are used principally to distinguish the species of *Stachybotrys* and *Memnoniella*.

THE PERFECT STATE

Melanopsamma pomiformis (Pers. ex Fr.) Sacc. is the only ascomycetous fungus known to have a *Stachybotrys* conidial state. This relationship was established by earlier workers on the basis of the association of conidia and perithecial stromata in nature. According to Booth (1957), the first cultural verification was made in 1944 by E. W. Mason who found *Stachybotrys socia* Sacc. from single ascospore cultures of *M. pomiformis*. Booth further confirmed Mason's cultural evidence and discussed the taxonomic dispositions of this fungus, including perfect and imperfect states, in detail. The pertinent literature dealing with this relationship is summarized briefly and chronologically as follows:

1805 Albertini and Schweinitz first reported the possible conidial state of *Sphaeria pomiformis* Pers.

1871 Berkeley and Broome described *S. pomiformis* with the conidial state *Sporocybe albipes* Berk. & Br.

1875 Saccardo created a new name *Fuckelina socia* Sacc. for the conidial state of *Eriosphaeria raripila* Sacc. which was later identified by Saccardo (1877) with *Sphaeria pomiformis*.

1822 Saccardo combined *Melanopsamma pomiformis* with *Sporocybe albipes* as its conidial state and placed *F. socia* as a synonym.

1886 Saccardo described *F. socia* with a note that it was a conidial state of *M. pomiformis* and possibly identical with *S. albipes*.

1909 Ferraris transferred *Fuckelina socia* to *Stachybotrys* and proposed the new combination *Stachybotrys socia* (Sacc.) Sacc.

1912 Ferraris listed *Stachybotrys socia* as the conidial state of *Melanopsamma pomiformis*.

1923 Höhnel found *Gliobotrys alboviridis* Höhnel (1902) associated with perithecia of *M. pomiformis* and identified the former with *Sporocybe albipes* and *Fuckelina socia*. A new combination *Fuckelina albipes* (Berk. & Br.) Höhnel was made.

1957 Booth gave a detailed account of the conidial state of *M. pomiformis* with the description and illustration from single ascospore cultures. The disposition of the conidial state was discussed. He considered *Sporocybe albipes* (=*Fuckelina albipes*), *Fuckelina socia* (=*Stachybotrys socia*), and *Gliobotrys alboviridis* synonyms, but did not make a new combination.

1958 Hughes listed *Stachybotrys socia* as the conidial state of *M. pomiformis*.

1968 Verona and Mazzucchetti reproduced Booth's description and drawing of the conidial state of *M. pomiformis* under the name *Stachybotrys socia*.

No perfect states in the life history of any species of *Memnoniella* have been reported.

KEY TO THE SPECIES OF *STACHYBOTRYS* AND *MEMNONIELLA* IN CULTURE

(Based on cultures grown on cornmeal agar except *S. theobromae* which is on potato dextrose agar)

1. Phialoconidia in chains (*Memnoniella*). 2
1. Phialoconidia in mucilaginous masses (*Stachybotrys*) 3

2. Mature conidia mostly 3-6 µm in diameter . . . . . *M. echinata*
2. Mature conidia mostly 6-9 µm in diameter . . . . . *M. subsimplex*

3. Mature conidia colorless . . . . . . *S. bisbyi*
3. Mature conidia green or olive gray 4

4. Mature conidia green, more than 12 µm in width . . . . *S. theobromae*
4. Mature conidia olive gray, less than 9 µm in diameter 5

5. Surface of mature conidia smooth-walled . . . . *S. albipes*
5. Surface of mature conidia coarsely roughened or striated or banded and ridged 6

6. Mature conidia spherical . . . *S. microspora*
6. Mature conidia not spherical 7

7. Surface of mature conidia with striation . . . . *S. cylindrospora*
7. Surface of mature conidia coarsely roughened, without striation 8

8. Mature conidia 5-6 X 3-3.5 µm . *S. parvispora*
8. Mature conidia larger, more than 7 µm in length and 4 µm in width 9

9. Mature conidia reniform or ellipsoidal to reniform 10
9. Mature conidia ovate to ellipsoidal 11

10. Mature conidia reniform, 10-12 X 5-6 µm . . . . *S. nephrospora*
10. Mature conidia ellipsoidal to reniform, 9-10 X 5-7 µm . . *S. oenanthes*

11. Mature conidia showing banded or ridged roughenings . . . . *S. chartarum*
11. Mature conidia not as above 12

12. Mature conidia ovate, 7.5-10 X 5-7 µm . . . . *S. dichroa*
12. Mature conidia ellipsoidal, 10-14 X 6-7 µm . . . . *S. kampalensis*

DESCRIPTIONS AND ILLUSTRATIONS

Stachybotrys albipes (Berk. & Br.) Jong & Davis, comb. nov.

≡ *Sporocybe albipes* Berk. & Br., Ann. Mag. Nat. Hist. 8: 19. 1971.

≡ *Fuckelina albipes* (Berk. & Br.) Höhnel, Zbl. Bakt. Bd. 60, p. 13. 1923.

= *Fuckelina socia* Sacc., Nuovo G. Bot. Ital. 7: 326. 1875.

≡ *Stachybotrys socia* (Sacc.) Sacc. in Ferraris, Ann. Mycol. 7: 283. 1909.

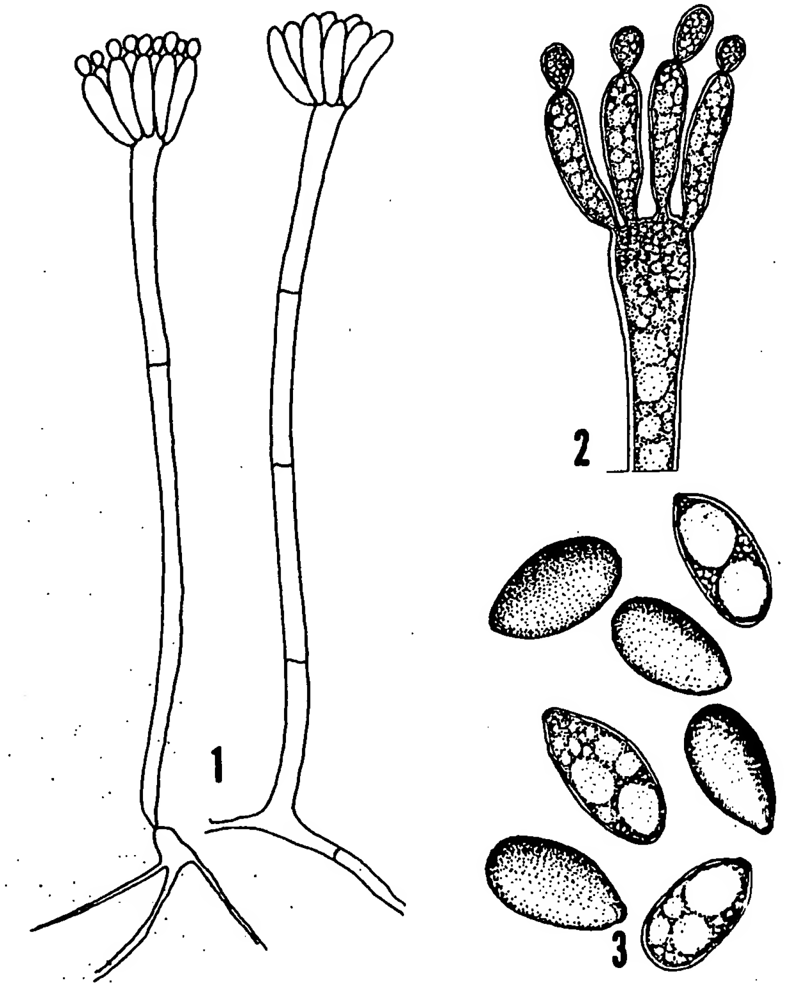
= *Gliobotrys alboviridis* Höhnel, S. B. Akad. Wiss. Wien, Abt. 1, 111: 1048. 1902.

Figures 1-6.

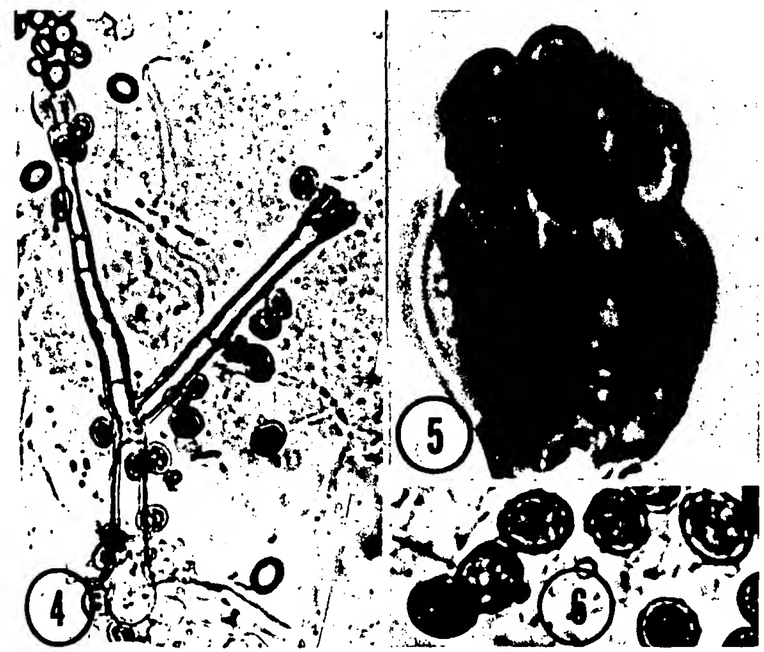
Growth on cornmeal agar extremely restrictied, attaining a diameter of 2 cm in 3 weeks. The surface of colony downy, uniformly dense, white, becoming dark granulate as conidia develop. Margin of colony distinct due to the absence of conidia, colorless, with compact submerged hyphae. No staining of medium in advance of mycelium. Reverse at first uncolored, later becoming olive green. Conidia produced abundantly a week following inoculation, first in the center, then centrifugally toward the margin of colonies.

Conidiophores determinate, macronematous, singly or in small groups, erect, straight or slightly curved, simple, occasionally branched, 3-7 µm wide, the basal cell slightly inflated and attenuate toward the tip, usually smooth throughout the length, but sometimes minutely rough-walled at the upper parts, slightly enlarged at the apex which bears terminal phialides in a whorl of 6-10 around a central phialide.

Phialides enteroblastic, determinate, discrete, unicellular, subclavate, hyaline, smooth-walled, 9-16 X 3-5 µm, with inconspicuous collarettes.



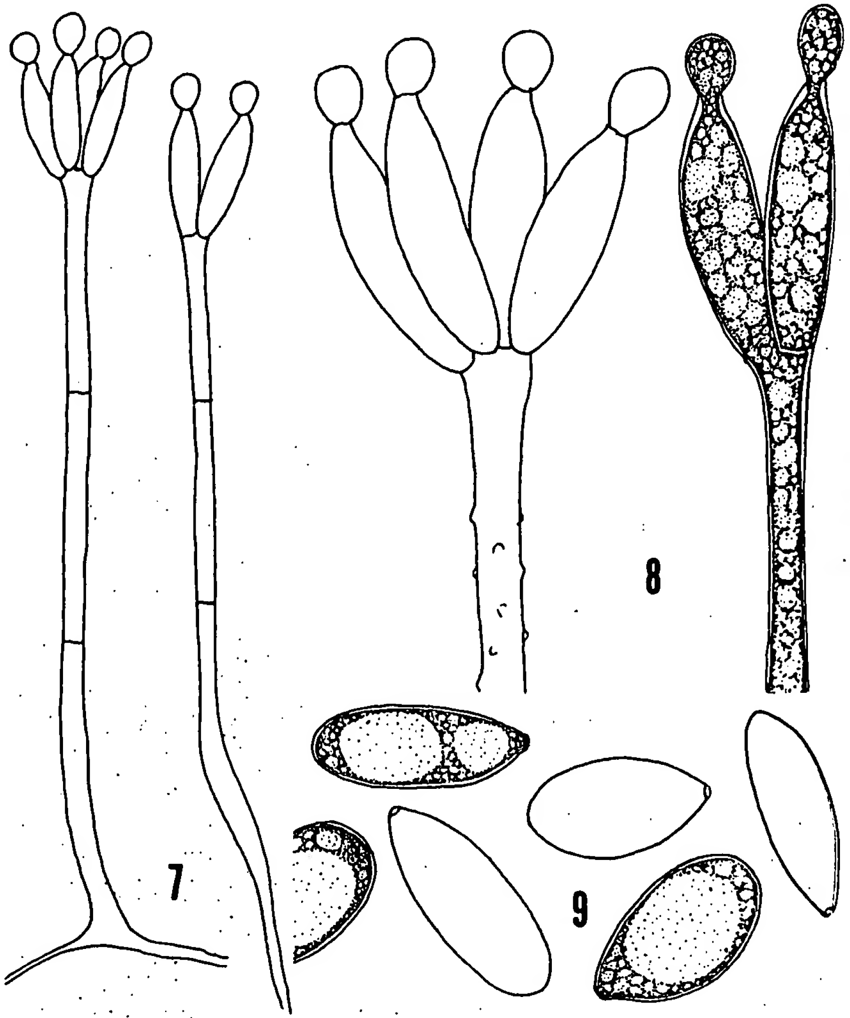
Figures 1-3. *Stachybotrys albipes* ATCC 18873.
1. Conidiophores with terminal phialides and phialoconidia. ca. X 500. 2. Phialides in a whorl of 4. ca. X 1,200.
3. Phialoconidia with a smooth surface. ca. X 2,400.



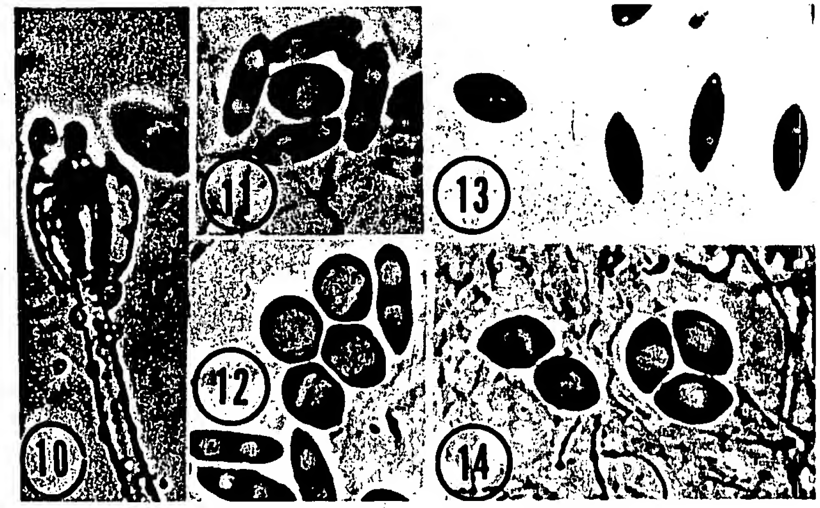
Figures 4-6. *Stachybotrys albipes* ATCC 18873.
4. Conidiophores with phialides and phialoconidia. ca. X 250. 5. Phialides with phialoconidia. ca. X 2,000.
6. Phialoconidia. ca. X 1,200.

Phialoconidia acrogenous, aggregated in slimy masses, unicellular, at first hyaline, when mature, dark olive gray, smooth-walled, ovate, size variable, 4-12 X 3-6 µm, mostly 7-9 X 5-6 µm.

REMARKS: The perfect state of this fungus is known to be *Melanopsamma pomiformis* (Pers. ex Fr.) Sacc. Booth (1957) has discussed the taxonomic dispositions of this fungus, including perfect and imperfect states, in detail. As the summary of the dispositions of the conidial state listed above, it appears that the name *Stachybotrys socia* is evidently preceded by *Sporocybe albipes* Berk. & Br. Since



Figures 7-9. *Stachybotrys bisbyi* ATCC 22173.
7. Conidiophores with phialides and phialoconidia. ca. X 800. 8. Phialides. ca. X 2,000. 9. Phialoconidia. Note the size and shape. ca. X 2,000.



Figures 10-14. *Stachybotrys bisbyi* from cotton blue in lactic acid mounts. 10. Phialides (ATCC 18825). ca. X 1,000. 11-14. Phialides. Note the size and shape. ca. X 1,000. 11. ATCC 18874. 12. ATCC 22215. 13-14. ATCC 22173.

Booth did not make a new combination, *Stachybotrys albipes* (Berk. & Br.) Jong & Davis is herein given in accordance with Articles 33 and 59 of the International Code of Botanical Nomenclature (Stafleu *et al.*, 1972).

The culture (ATCC 18873) studied is a single ascospore isolate of *M. pomiformis* which was originally isolated by Booth (1957) from *Ulmus* sp. in Fountains Abbey Woods, Yorkshire. This culture was received from Commonwealth Mycological Institute (CMI) as *Melanopsamma pomiformis* (Pers. ex Fr.) Sacc. IMI 56,393. It produces only a *Stachybotrys* state in culture. Growth and sporulation occur on cornmeal agar at temperatures in the range of 15 to 30 C. It grows on cellulose agar, but no clearing of the medium indicates that it is not a cellulose-decomposing strain. Morphologically,

Stachybotrys albipes is akin to *S. dichroa* in having thick-walled simple conidiophores, but they can be distinguished by the ornamentation of the spore wall on which the former is smooth and the latter is coarsely roughened.

Stachybotrys bisbyi (Srinivasan) Barron, Mycologia 56: 315. 1964.

≡ *Hyalostachybotrys bisbyi* Srinivasan, J. Indian Bot. Soc. 37: 341. 1958.

= *Hyalostachybotrys sacchari* Srinivasan, J. Indian Bot. Soc. 37: 341. 1958.

≡ *Stachybotrys sacchari* (Srinivasan) Barron, Mycologia 56: 315. 1964.

= *Stachybotrys aurantia* Barron, Can. J. Bot. 40: 258. 1962.

Figures 7-14.

Colonies on cornmeal agar covering Petri dish plates in 2 weeks. The surface downy, uniformly dense, white, covered by a pale gray to salmon pink powdery bloom of conidial masses. Margin of colony not distinct, with compact hyphae. No staining of medium in advance of mycelium. Conidia produced abundantly a week after inoculation of plates. Cultures tending to lose the ability to produce conidia after several transfers.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple or branched, 2-5 septate, hyaline, up to 200 µm long, 3-4 µm wide, the basal cell slightly inflated, attenuate toward the tip, sometimes minutely rough-walled at the upper parts, sometimes more or less smooth throughout the length. slightly enlarged at the apex which bears terminal phialides in a whorl of 3-8 around a central phialide.

Phialides enteroblastic, determinate, discrete, unicellular, subclavate, hyaline, smooth-walled, 10-17 X 4-6 µm, with conspicuous collarettes.

Phialoconidia acrogenous, aggregated in slimy masses, hyaline, smooth-walled, one-celled, one to three guttulate, lemon-shaped or fusiform, 8-14 X 6-9 µm or 10-16 X 3-6 µm.

REMARKS: Bisby (1943) first reported a hyaline form of *Stachybotrys* isolated from soil by J. E. Machacek in Winnipeg, Canada. He called it a "pink *Stachybotrys*." When he knew Machacek later found a few more similar cultures from soil in Manitoba, Bisby (1945) suggested that it might represent a new genus and species.

Srinivasan (1958) concurred with Bisby's suggestion and created a new genus *Hyalostachybotrys* to accommodate these hyaline fungi resembling *Stachybotrys*. He described two species based on his own isolates from tropical South India. The type species, *H. bisbyi*, was isolated from rhizosphere of *Erianthus munja* Michaux and of *E. arundinaceus* Michaux. The "pink *Stachybotrys*" (IMI 10945) originally isolated from soil by Machacek was also disposed as *H. bisbyi*. The second species, *H. sacchari*, was a weak pathogen of sugar cane sheaths and was also isolated from the rhizosphere of sugarcane. The delimitation of these two species was primarily based on size and shape of phialospores. Srinivasan also clearly indicated that there were variations among the cultures studied by him in the amount of aerial mycelium, color of spore masses, branching of the conidiophores and in the size and shape of conidia.

Unaware of Srinivasan's work, Barron (1926) described a new soil hyphomycete, *Stachybotrys aurantia* Barron, which is identical with Srinivansan's *H. bisbyi*. Barron (1964) later disagreed with Srinivasan's creation of the genus *Hyalostachybotrys* because he believed that a genus based on color of the conidium would have no taxonomic validity. He therefore relegated *Hyalostachybotrys* to synonymy with *Stachybotrys*, and considered both of Srinivasan's species as *Stachybotrys*.

In the present study the following hyaline *Stachybotrys* isolates were studied.

ATCC 18825: received from L. W. Durrell, Colorado State Univ. (as *S. bisbyi*), isolated by W. A. Kreutzer from rhizosphere of Western wheat grass, Society Islands.

ATCC 18850 = IMI 100,544 (as *S. sacchari*): isolated by B. L. Mathur, from soil, India.

ATCC 18853 = IMI 116,426 (as *S. sacchari*): isolated by A. H. Moubasher from soil, Egypt.

ATCC 18874 = IMI 91,211 (as *S. sacchari*): isolated by J. R. Anderson from *Saccharum officinarum*, S. Africa.

ATCC 18885 = IMI 10,945 (as *S. bisbyi*): isolated by Machacek from soil, Canada.

ATCC 22172 = CBS 363.58 (as *S. bisbyi*): isolated by H. J. Swart from soil mangrove swamps, Mozambique.

ATCC 22173 = CBS 399.65 (as *S. bisbyi*): isolated by I. Focke from *Zea mays* root.

ATCC 22215: received from M. Morrall, Univ. of Saskatchewan (as *Stachybotrys* sp.), isolated from roots of grasses, Canada.

ATCC 22701: isolated by T. Matsushima from rotton wood, Papua-New Guinea (as *S. aurantia* MFC-2833).

ATCC 22702: isolated by T. Matsushima from soil, Papua-New Guinea (as *S. aurantia* MFC-4097).

• Because of the extreme variability in the conidial morphology of these isolates, a clear differentiation of *S. bisbyi* and *S. sacchari* seems impossible. According to Srinivasan (1958), the size of conidia is 10.0-16.4 X 5.0-11.6 µm for *S. bisbyi* and 7.6-11.6 X 2.6-3.7 µm and 6.5-10.8 X 5.3-8.5 µm for *S. sacchari*. However, the description and illustration of *S. bisbyi* (as *S. aurantia*) given by Barron (1962) shows that it also properly fits the description and illustration of *S. sacchari* given by Srinivasan. It is apparent that in the past the concept

of both species is overlapped and possibly one is the morphological variant of the other.

The species description presented is primarily based upon the strain ATCC 22173. Indeed, all the strains examined produce both globose and fusiform types of conidia, but most often they are dominated by one type depending on culture conditions such as media, temperature, light and age of cultures. The conidial size, 10-16 X 3-6 µm for fusiform and 8-14 X 609 µm for lemon-shaped, includes those of *S. bisbyi* and *S. sacchari* originally described by Srinivasan. Since *S. bisbyi* is the type species of the genus *Hylostachybotrys* and has a priority over *S. sacchari*, it is herein recommended that *S. sacchari* be considered a synonym of *S. bisbyi*.

Two additional hyaline species were described by Rifai (1964, 1974) from Java. *Stachybotrys bambusicola* Rifai was recovered on the fallen culm sheath of cultivated bamboo and *S. palmijunci* Rifai was on a decaying stem of the rattan *Daemonorhops melanochaetes* Bl. In both species the conidiophores are reddish brown and capable of elongating by proliferations; the phialoconidia are hyaline or subhyaline but pink in masses. They have only been recorded from the type locality and there are no cultures available for further studies. According to Rifai (1974), the two Javanese species are closely related but differ in size and shape of phialoconidia.

Stachybotrys chartarum (Ehrenb. ex Link) Hughes, Can. J. Bot. 36: 812. 1958.

≡ *Stilbospora chartarum* Ehrenb., Sylvae Myc. Berol. pp. 9, 21. 1818.

≡ *Oidium chartarum* Ehrenb. ex Link, Linn. Spec. Plant., IV, 6(1): 124. 1824.

≡ *Oospora chartarum* (Ehrenb. ex Link) Wallr., Flora Crypt. German. 2: 184. 1833.

• *Stachybotrys atra* Corda, Icon. Fung. 1: 21. 1837.

• *Aspergillus alternatus* Berk., Ann. Mag. Nat. Hist. 1: 262. 1838.

= *Sporocybe lobulata* Berk., Ann. Mag. Nat. Hist. 6: 434. 1841.

≡ *Stachybotrys lobulata* (Berk.) Berk., Outlines Brit. Fung. p. 343. 1860.

= *Synsporium biguttatum* Preuss, Klotzschii Herb. Viv. Mycol., No. 1285, anno 1849.

= *Stachybotrys alternans* Bon., Handb. Myk. 117. Fig. 185. 1851.

= *Memnonium sphaerospermum* Fuckel, Symb. Mycol. p. 358. 1870.

= *Stachybotrys scabra* Cooke & Harkness, Grevillea 12: 96. 1884.

= *Stachybotrys asperula* Massee & Cooke, Grevillea 16: 26. 1887.

= *Stachybotrys verrucosa* Cooke & Massee, Grevillea 16: 102. 1888.

= *Stachybotrys atrogrisea* Ellis & Ev., J. Mycol. 4: 106. 1888.

= *Trichosporum effusum* (Corda) Sacc. subsp. *binucleatum* Karst., Hedwigia 29: 272. 1890.

= *Stachybotrys gracilis* Em. March., Bull. Soc. Belg. Micro. 7: 265. 1894.

= *Stachybotrys pulchra* Speg., Rev. Agr. y Veter. La Plata. p. 248. 1896.

= *Stachybotrys elasticae* Koord., Botan. Untersuch. Java Pilza. p. 227. Fig. 37. 1907.

= *Stachybotrys dakotensis* Sacc., Atti Mem. R. Acad. Sci., Lett., Arti, Padova. 33: 174. 1917.

= *Stachybotrys voglinii* Cif., Ann. Mycol. 20: 48. 1922.

Figures 15-20.

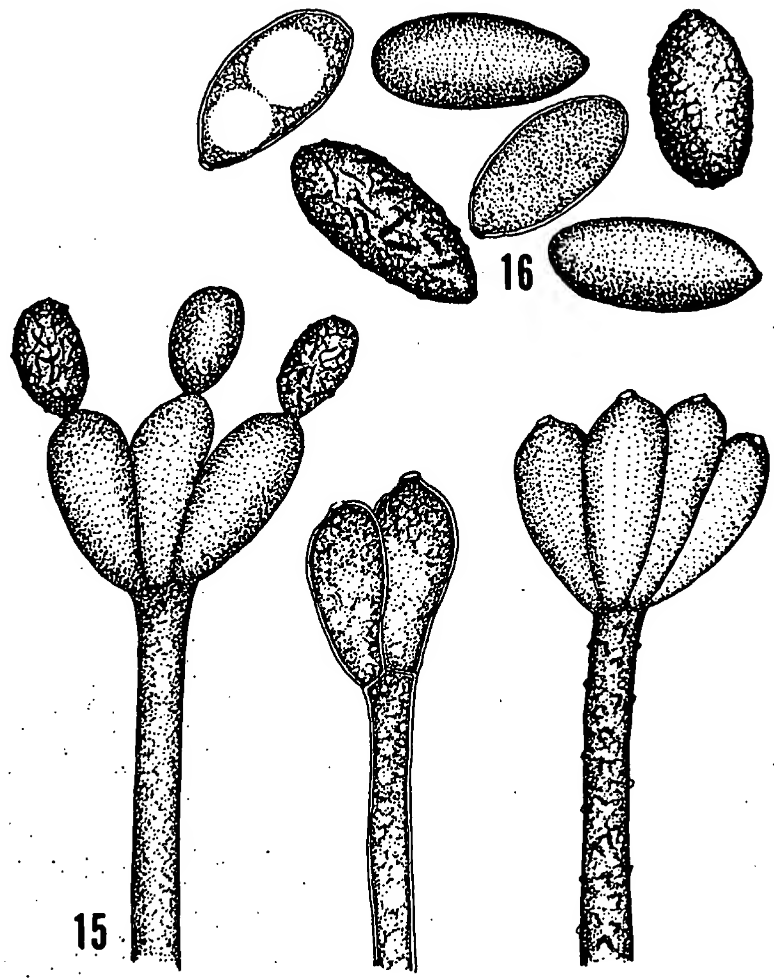
Colonies on cornmeal agar covering Petri-dish plates in 3 weeks, appressed, uniformly dense, uncolored at first, becoming dull dark, covered by a dark powdery bloom of conidial masses. Margin of colony distinct due to the absence of conidia, white, with compact hyphae. No staining of medium in advance of mycelium. Conidial production in abundance 3 days following inocluation on the plates.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple or irregularly branched, 2-4 septate, hyaline at the base, dark olivaceous toward the apex, length variable, up to 1,000 µm long, 3-6 µm wide, the basal cell slightly inflated, sometimes minutely rough-walled at the upper parts, sometimes more or less smooth throughout the length, slightly enlarged at the apex which bears terminal phialides in a whorl of 3-9 around a central phialide.

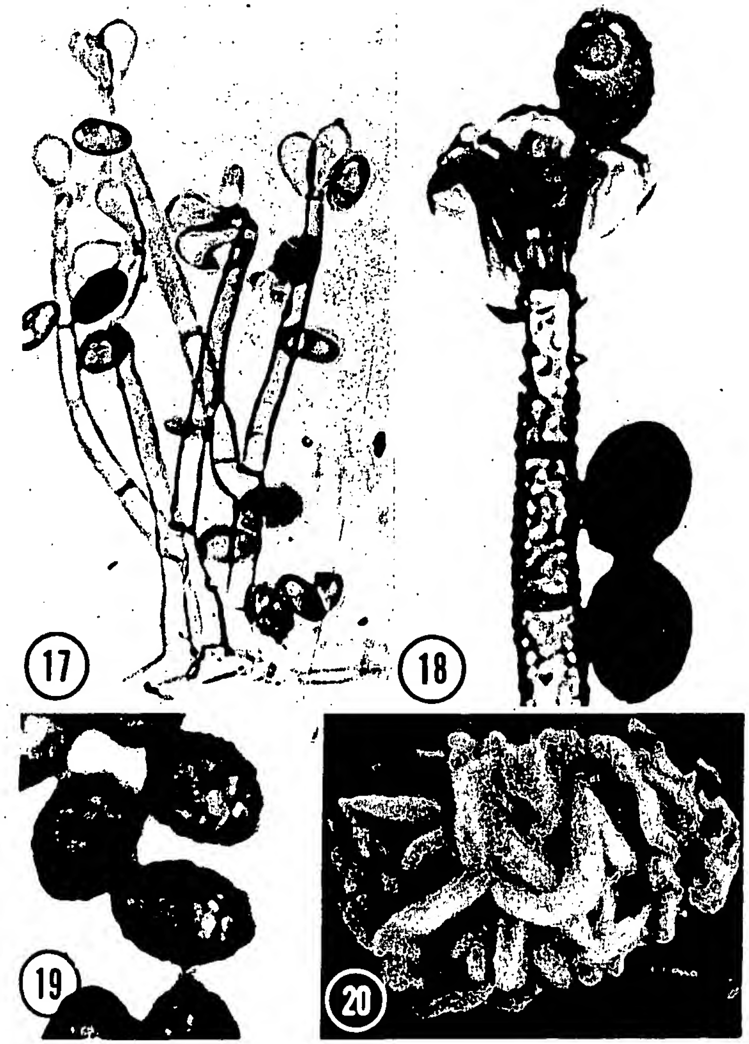
Phialides enteroblastic, determinate, discrete, unicellular, at first hyaline, later dark olivaceous, obovate or ellipsoid, smooth-walled, 9-14 X 4-6 µm, with conspicuous collarettes.

Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline, when mature, dark olive gray, more or less opaque, smooth-walled or showing banded or ridged, ellipsoidal, unicellular, 7-12 X 4-6 µm.

REMARKS: *Stachybotrys atra*, the type species of the genus *Stachybotrys*, was first described by Corda (1837) with two-celled conidia. From critical studies of cultures and herbarium specimens, Bisby (1943) emended both generic and species descriptions with one-celled phialoconidia. Because of the great variability in the morphology of *S. atra* examined in culture, Bisby reduced more than ten *Stachybotrys* species which he thought were based on such morphological variants to synonyms with *S. atra*. Hughes (1958) later reexamined the type material of *S. atra* and identified it with *Stachybotrys chartarum* (Ehrenb.) Hughes. However, the correct combination should be *S. chartarum* (Ehrenb. ex Link) Hughes, in accordance with Article 13 of the International Code of Botanical Nomenclature (Stafleu *et al.*, 1972).



Figures 15-16. *Stachybotrys chartarum* ATCC 11716. 15. Phialides. Note the conspicuous terminal openings through which phialoconidia are being produced. ca. X 1,500. 16. Phialoconidia. Note the ridged or banded surface. ca. X 2,500.



Figures 17-20. *Stachybotrys chartarum* ATCC 11716. 17. Branching habit of conidiophores. ca. X 1,000. 18. Phialides. ca. X 2,000. 19-20. Phialoconidia featuring a ridged or banded surface. 19. Regular light micrograph. ca. X 2,000. 20. Scanning electron micrograph. ca. X 6,000.

The synonyms listed above are compiled from opinions of Bisby (1943) and Hughes (1958).

The following cultures were studied and are considered to be *S. chartarum*.

ATCC 9182 = NRRL 1877 = QM 1274 (as *S. atra*): isolated by W. Crozier from paper, Washington, D. C.; used in United States for testing mildew proofing.

ATCC 11695 = QM 94d (as *S. atra*): isolated by E. T. Reese from Trousers, New Guinea; used in cellulose and wool decomposition.

ATCC 11716 = IFO 5369 (as *S. lobulata*): isolated by Y. Sasaki (F-14) from coal, Japan.

ATCC 16026 = IMI 82,021 (as *S. atra*) = QM 8401: isolated by R. M. Everett from cotton fabric, U. K.; meets British specifications for fungus resistance tests.

ATCC 18541: isolated by Carol A. Shearer from balsa wood submerged in Patuxent River, Maryland.

ATCC 18836 = IFO 7222: isolated by K. Tubaki (TC-63-1), Japan.

ATCC 18842 = CBS 222.46 (as *S. atra*): isolated by M. B. Bok from flax fibre.

ATCC 18843 = CBS 324.65 (as *S. atra*): isolated by CBS staff from tile.

ATCC 18844 = CBS 328.37 (as *S. atra* var. *brevicaulis*): isolated by O. Verona from paper.

ATCC 18845 = CBS 329.37 (as *S. atra* var. *genuina*): isolated by O. Verona from paper.

ATCC 18846 = CBS 330.37 (as *S. atra* var. *lobulata*): isolated by O. Verona from paper.

ATCC 18847 = CBS 341.35 (as *S. atra*): isolated by N. F. Conant.

ATCC 18875 = IMI 42,310 (as *S. atra*): isolated in Peshawa, India.

ATCC 18979: isolated by O. Fassatiova (as *S. alternans* CCF 584).

ATCC 22127: isolated by D. Mallock from woodchuck dung.

ATCC 22218 = IMI 136,344 (as *S. atra* var. *microspora*): isolated by C. V. Subramanian from soil, India.

ATCC 22703: isolated by T. Matsushima (MFC-2762) from soil, Goroka.

ATCC 26303 = QM 1533 (as *S. atra*): isolated by W. L. White from old cardboard dumped in wasteland; produces 12,13-epoxy-Δ^9-trichothecenes as the mycotoxins responsible for stachybotryotoxicosis (Eppley & Bailey, 1973).

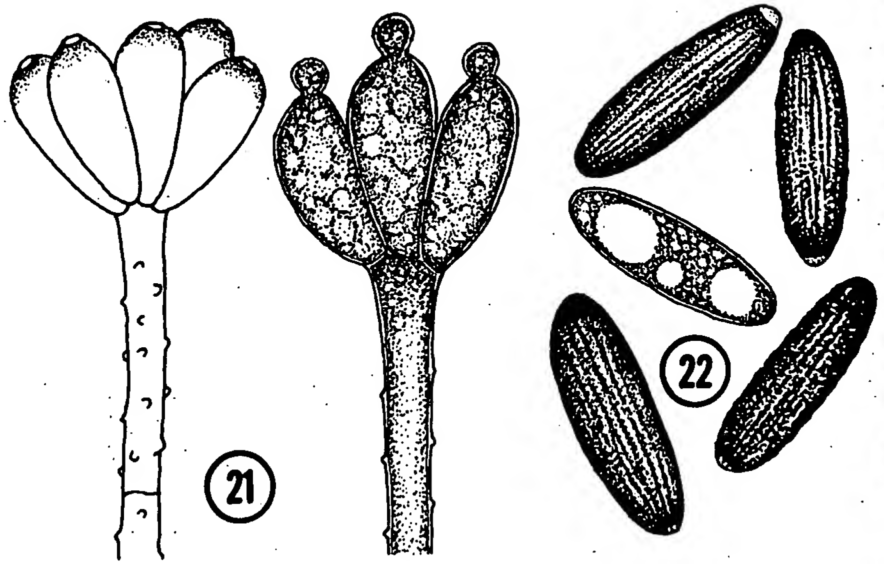
ATCC 26384: isolated by A. Ylimaki from barley, Finland; agent of stachybotryotoxicosis of laboratory animals (Korpinen, 1973).

ATCC 26385: isolated by A. Ylimaki from wheat, Finland; agent of stachybotryotoxicosis of laboratory animals (Korpinen, 1973).

ATCC 26386: isolated by A. Ylimaki from commercial pig feed, Finland; agent of stachybotryotoxicosis of laboratory animals (Korpinen, 1973).

The distinguishing feature of this species is the mature phialoconidia showing a ridged or banded surface and their size.

All the strains studied grow and sporulate well on CMA and rabbit food agar at temperatures in the range of 10 to 37 C. The upper limit of temperature for spore germination is also 37 C. Production of clearing by growth on cellulose agar medium indicates that it is a strong cellulose-decomposing fungus. ATCC 9182 and ATCC 16026 are being used for fungus resistance tests in U. S. and British military specifications.



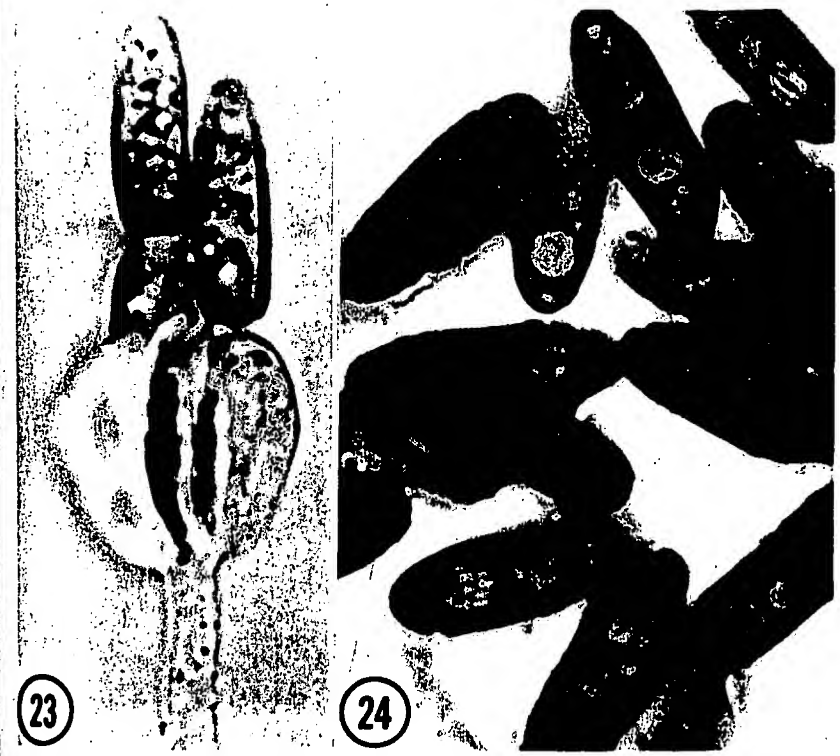
Figures 21-22. *Stachybotrys cylindrospora* ATCC 18851. 21. Phialides. ca. X 2,000. 22. Phialoconidia. Note striations running along the length of conidia. ca. X 2,000

Stachybotrys cylindrospora Jensen, Cornell Univ. Agr. Exp. Sta. Bull. 315: 496. 1912.

≡ *Stachybotrys atra* Corda var. *cylindrospora* (Jensen) Rayss & Borut, Mycopath. Mycol. Appl. 10: 168. 1956.

Figures 21-24.

Colonies on cornmeal agar covering Petri-dish plates in 3 weeks, downy to felty, colorless at first, becoming dark, with a granulate surface as conidial production commences. Margin of colony not distinct, with compact hyphae and very few conidia. Reverse stained yellowish pink. Conidia produced in abundance a week after inoculation of plates.



Figures 23-24. *Stachybotrys cylindrospora* ATCC 18851. 23. Phialides. ca. X 2,000. 24. Phialoconidia showing delicate striations. ca. X 2,000.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple or irregularly branched, 3-5 septate, hyaline at the base, slightly olivaceous toward the apex, length variable, up to 200 μm long, 3-5 μm wide, the basal cell slightly inflated, attenuate toward the tip, sometimes minutely rough-walled at the upper parts, sometimes more or less smooth throughout the length, slightly enlarged at the apex which bears terminal phialides in a whorl of 3-9 around a central phialide.

Phialides enteroblastic, determinate, discrete, unicellular, subclavate, dark olivaceous at the tip, smooth-walled, 11-16 X 4-5 μm, with conspicuous collarettes.

Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline and smooth-walled, when mature, dark olive gray, surface showing delicate striations running obliquely along the length of the conidia, cylindrical, long and narrow, 2 to 4 times as long as broad, unicellular, 13-16 X 4-6 µm, usually containing two oil drops.

REMARKS: *Stachybotrys cylindrospora* was first discovered by Jensen in 1912 from soil in New York state. Since the original description of this species showed so much resemblance to that of *S. chartarum* (as *S. atra*), Bisby (1943) suggested that they were possibly synonymous. Rayss and Borut (1956) later recognized the fungus as a variety of *S. chartarum* (as *S. atra*) mainly by the phialoconidia which are cylindrical and thinner than in the typical variety. In studies of the flora of organic soil in Ontario, Canada, Barron (1961) obtained several isolates which agree well with Jensen's description and suggested that *S. cylindrospora* should be recognized as a distinct species.

The culture ATCC 16276 was isolated by W. Gams from wheat field soil in Kitzeberg, Germany, and determined by G. L. Hennebert as *S. cylindrospora*. ATCC 18851 (= IMI 85,334) was isolated and identified by G. L. Barron (1961) from peat soil in Quelph, Canada. Both strains studied appear to be quite distinctive from the type species *S. chartarum* in having phialoconidia, as the specific epithet indicates, long and narrow, and the surface featuring delicate striations running obliquely along the length of the conidia. The fungus is now known in Europe, Japan, North America, British Solomon Islands (Matsushima, 1971a; 1975), and is well established in different climate regions of the world. Thus, it has been shown that this species is a clearly defined fungus taxon.

The temperature range for growth and conidial germination of the fungus is from 15 to 30 C. No production of clearing by growth on the cellulose agar medium indicates that it is not a cellulose-decomposing fungus.

Stachybotrys dichroa Grove, J. Bot. Lond. 24: 201. 1886.

Figures 25-30.

Growth on cornmeal agar extremely restricted, attaining a diameter of 2 cm in 3 weeks. The surface of colony downy, orange, with little or no aerial mycelium. Margin of colony not distinct, with compact hyphae. Reverse stained bright orange. Conidial production usually sparse, confined to the central part of the colony. Cultures tending to lose their ability to produce conidia after several transfers.

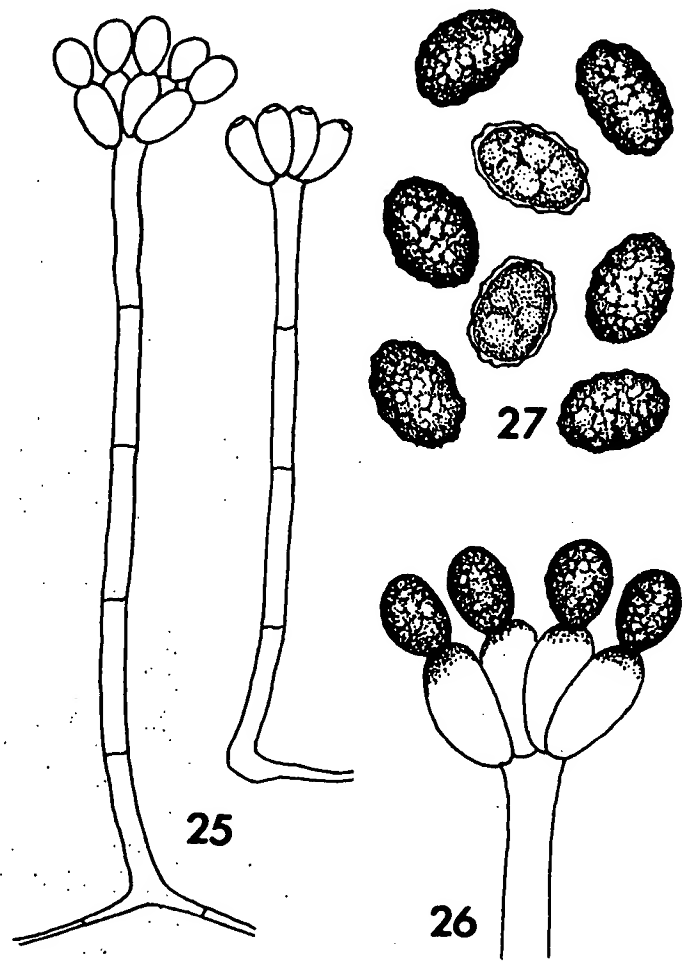
Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple, 2-7 septate, hyaline, thick-walled, lenght variable, up to 210 µm long, 4-9 µm wide, the basal cell slightly inflated, attenuated toward the tip, usually smooth throughout the length, slightly enlarged at the apex which bears terminal phialides in a whorl of 4-6 around a central phialide.

Phialides enteroblastic, determinate, discrete, unicellular, subclavate, hyaline, smooth-walled, 8-10 X 5-6 µm, with conspicuous collarettes.

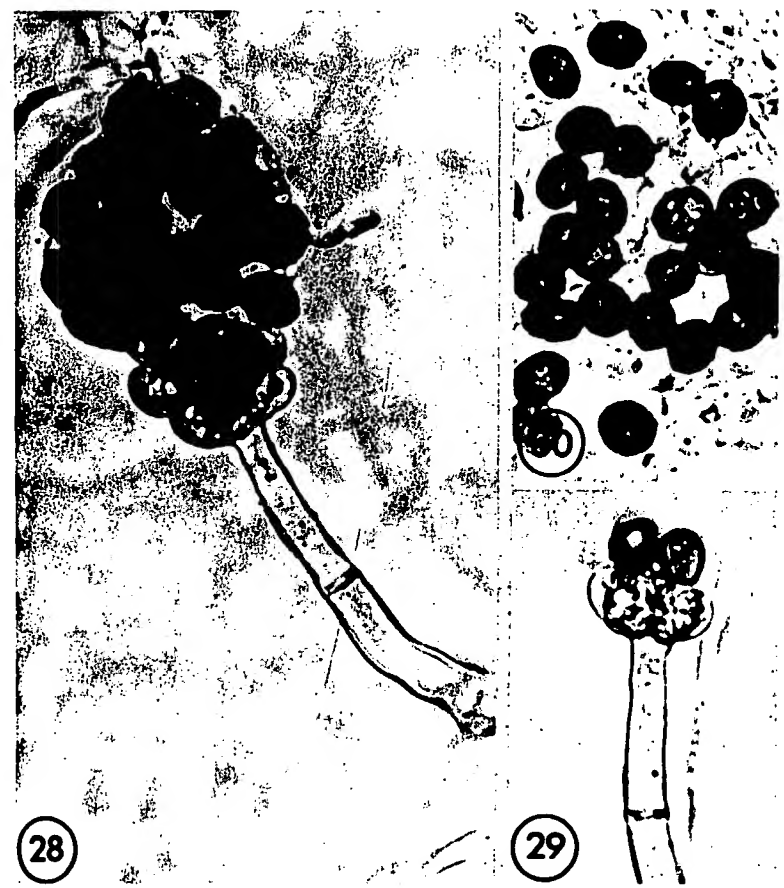
Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline, when mature, dark olive gray, more or less opaque, coarsely roughened, unicellular, ovate, 7.5-10 X 5-7 µm.

REMARKS: On examination of Grove's type material, Bisby (1943) first concluded that *S. dichroa* was young *S. chartarum* (as *S. atra*). However, Bisby and Ellis (1949) later recognized *S. dichroa* as a taxonomically distinct species after studying six collections from different localities, including two in culture. The fungus is easily distinguished by its habitat, cultural characteristics and the thick-walled simple conidiophores. According to Bisby and Ellis (1949), it is not uncommon on dead stems of herbaceous plants in England. It has not been found from soil.

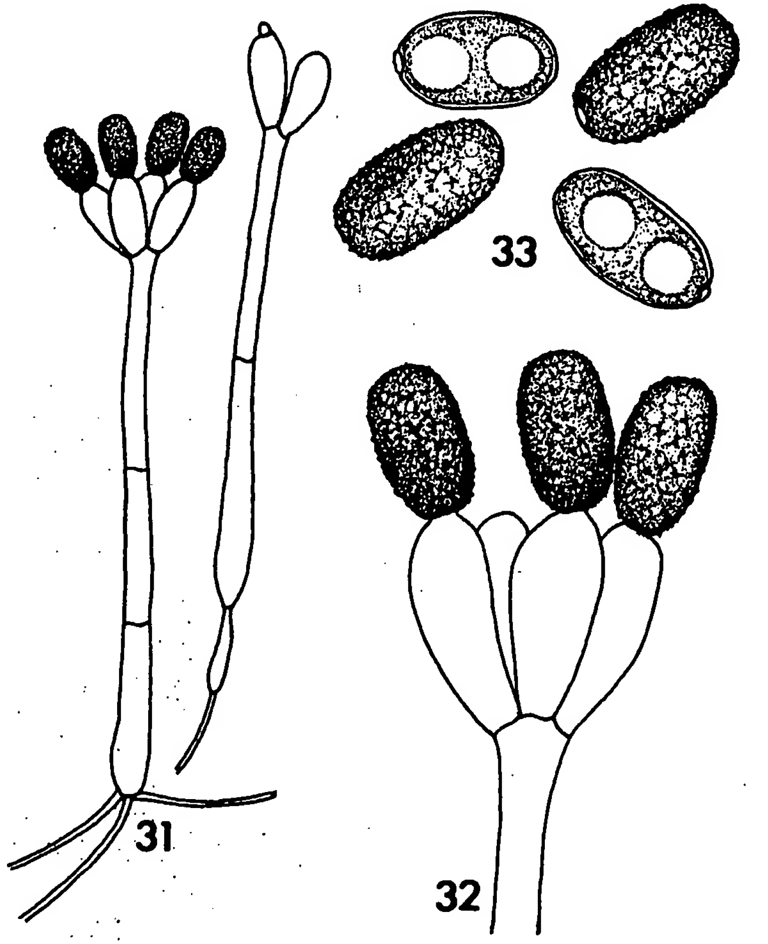
The culture ATCC 18913 (=IMI 17,506) studied was isolated by M. B. Ellis from a dead herbaceous stem and further studied by Bisby and Ellis (1949). It grows and sporulates on cornmeal agar at temperatures in the range of 15 to 26 C. The upper limit of temperature for spore germination is 30 C. Production of clearing by growth on cellulose agar medium indicates that it is a cellulose-decomposing fungus.



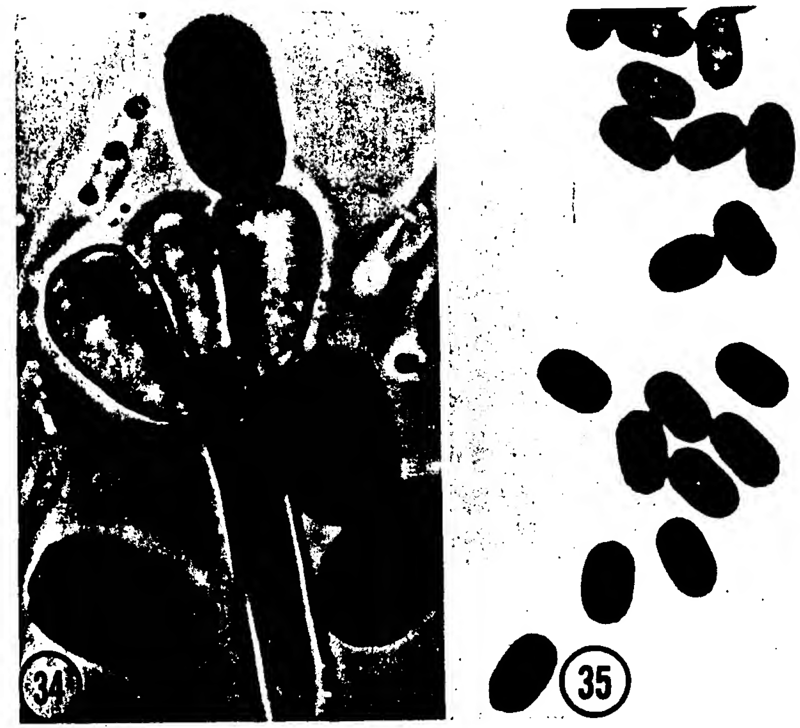
Figures 25-27. *Stachybotrys dichroa* ATCC 18913.
25. Conidiophores with phialides and phialoconidia. ca. X 500. 26. Phialides. ca. X 1,500. 27. Phialoconidia. ca. X 1,800.



Figures 28-30. *Stachybotrys dichroa* ATCC 18913.
28. Conidiophore with terminal phialides bearing a slimy mass of conidia. ca. X 1,000. 29. Phialides with phialoconidia. ca. X 1,000. 30. Mature phialoconidia featuring a coarsely roughened surface. ca. X 1,000.



Figures 31-33. *Stachybotrys kampalensis* ATCC 22705. 31. Conidiophores with phialides and phialoconidia. ca. X 800. 32. Phialides and phialoconidia. ca. X 2,000. 33. Phialoconidia. ca. X 2,000.



Figures 34-35. *Stachybotrys kampalensis* ATCC 22705. 34. Phialides and phialoconidia. ca. X 2,000. 35. Phialoconidia. ca. X 1,000.

Stachybotrys kampalensis Hansford, Proc. Linn. Soc. Lond. 155: 45. 1943.

Figures 31-35.

Growth on cornmeal agar somewhat restricted, reaching 3 cm in diameter in 2 weeks, appressed, uniformly dense, uncolored at first, becoming dull dark, covered by a dark bloom of conidial masses. Margin of colony distinct due to the absence of conidia, with compact hyphae. No staining of medium in advance of mycelium. Conidial production abundant 3 days following inoculation on the plates.

Conidiophores determinate, macronematous, usually solitary but occasionally in groups, straight or slightly

curved, simple, 1-3 septate, hyaline, thick-walled, length variable, up to 180 µm long, 4-8 µm wide, smooth throughout the length, the basal cell inflated, tapering toward the apex which bears terminal phialides in a whorl of 4-8 around a central phialide.

Phialides enteroblastic, determinate, discrete, unicellular, hyaline, obovate to ellipsoid, smooth-walled, 9-13 X 6-7 µm, with conspicuous collarettes.

Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline and smooth-walled, when mature, dark olivaceous to almost black, heavily verrucose, ellipsoidal, unicellular, 10-14 X 6-7 µm, usually containing two oil drops.

REMARKS: *Stachybotrys kampalensis* was first discovered by Hansford (1943) on dead wood in Uganda. Hughes (1952) later reported three additional collections from the Gold Coast on petioles of *Carica papaya* L. and stems of *Hibiscus esculentus* L. Matsushima (1975) also found this fungus in Japan on leaves of *Pueraiae hirsutae* and *Musae paradisiacae*.

The strain ATCC 22705 studied was isolated by Matsushima (1971) from forest soil in New Guinea. The strain ATCC 32255, a subculture of CBS 388.73, was isolated from *Euphorbia tirukalli*. The identity of both strains has been further confirmed by a comparative study with the Hansford's type material deposited at the Kew Herbarium (K), England. They grow and sporulate on cornmeal agar at temperatures in the range of 15 to 30 C. No production of clearing on the cellulose agar medium indicates that the fungus does not decompose cellulose.

Stachybotrys microspora (Mathur & Sankhla) Jong & Davis, comb. nov.

≡ *Stachybotrys atra* Corda var. *microspora* Mathur & Sankhla, Sci. Culture 32: 93. 1966.

Figures 36-40.

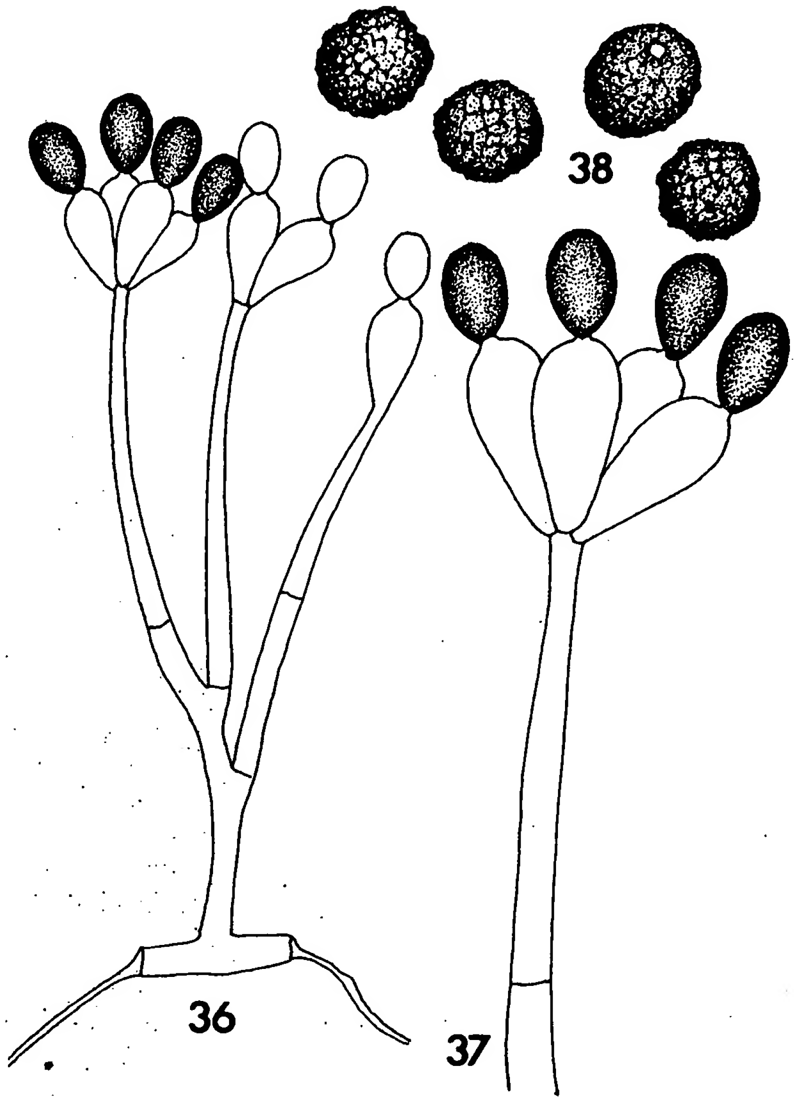
Colonies on cornmeal agar covering Petri-dish plates in 3 weeks, appressed, uniformly dense. Central parts of colonies becoming dull dark, covered by a dark powdery bloom of conidial masses. Margin of colonies not distinct, with compact hyphae. No staining of medium in advance of mycelium. Conidial production abundant, confined to the central part of the colony.

Conidiophores determinate, macronematous, solitary or in groups, irregularly dark olivaceous toward the apex, up to 55 µm long, 2-4 µm wide, usually smooth throughout the length, sometimes minutely rough-walled at the upper parts, the basal cell inflated, tapering toward the apex which bears terminal phialides in a whorl of 2-6 around a central phialide.

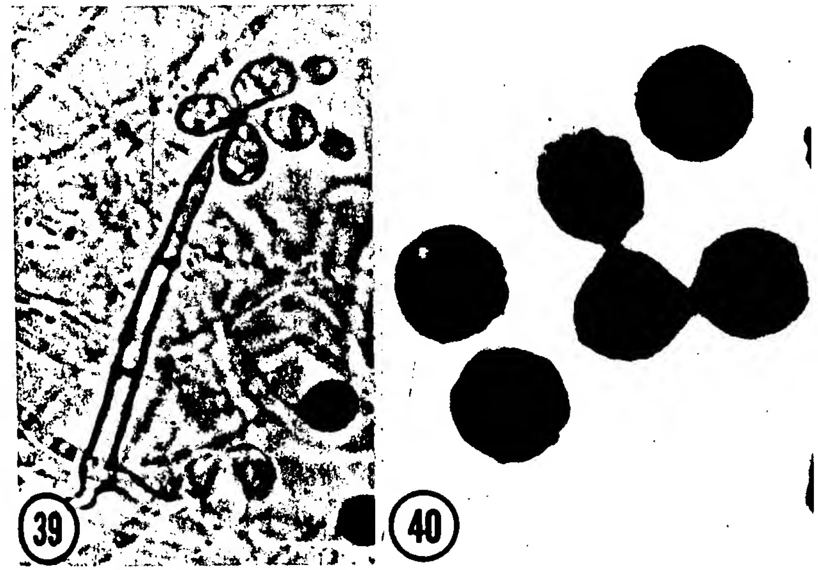
Phialides enteroblastic, determinate, discrete, clustered in a verticillate arrangement, unicellular, slightly dark olivaceous, obovate to pyriform, smooth-walled, 8-9 X 4-5 µm, with conspicuous collarettes, detachable from conidiophores.

Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, when young appearing as more or less elliptical or pyriform 6-8 X 4-5 µm, becoming globose, 5-6 µm in diameter at maturity, dark olive gray, more or less opaque, coarsely roughened, unicellular.

REMARKS: Mathur and Sankhla (1966) first discovered this fungus from soil in Jaipur, India, and described it as *Stachybotrys atra* Corda var. *microspora* Mathur & Sankhla. The dried type culture of this fungus was deposited in the herbarium of the Commonwealth Mycological Institute (IMI 91,933). An examination of this plate culture shows that it is mixed with *Stachybotrys chartarum*. Fortunately, each can be identified in accordance with its colony characteristics. A permanent slide made from a colony of *S. atra* var. *microspora* of this type material has been deposited in the herbarium of the National Fungus Collections (BPI), Beltsville, Maryland. The strain ATCC 18852 (=IMI 124,902) was isolated by D. McDonald from *Arachis hypogaea* rhizosphere in Zaria, N. Nigeria, in 1967. It is in full agreement with the type material. Since the culture characteristics, conidia and conidiophores are easily distinguished from those of *S. chartarum*, we would



Figures 36-38. *Stachybotrys microspora* ATCC 18852. 36. Branching habit of a conidiophore with phialides and phialoconidia. ca. X 1,500. 37. Phialides. ca. X 2,000. 38. Mature phialoconidia. ca. X 2,000.

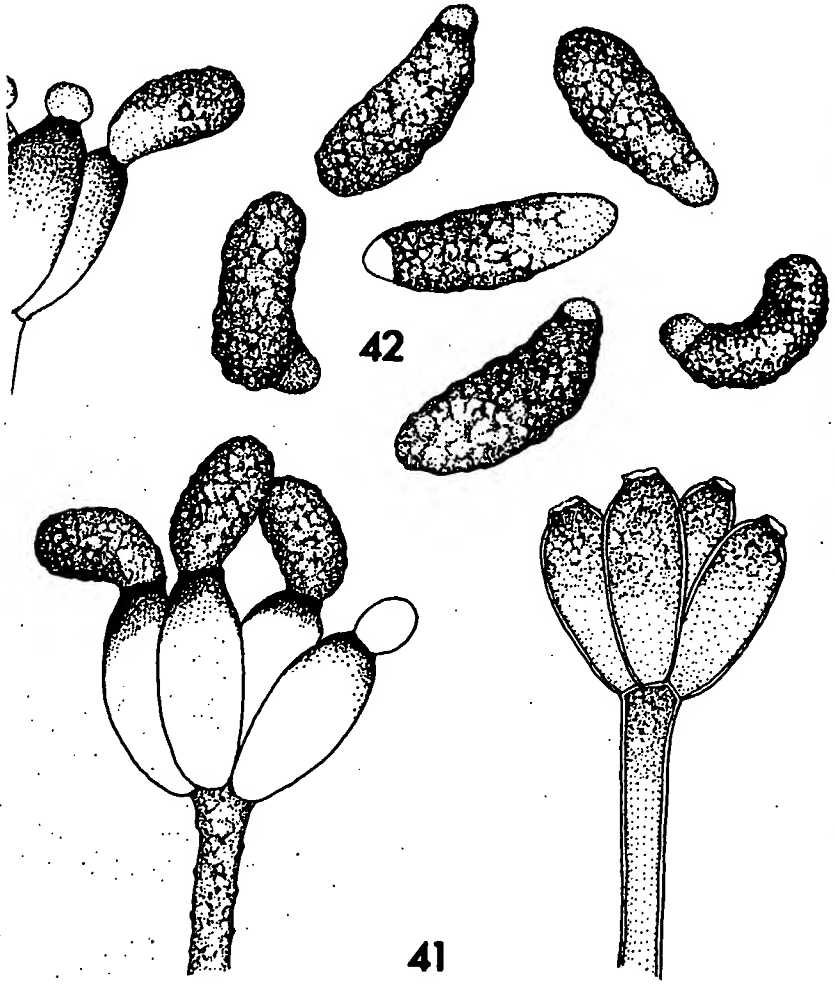


Figures 39-40. *Stachybotrys microspora* ATCC 18852. 39. Growth habit of a conidiophore. ca. X 1,000. 40. 40. Mature phialoconidia featuring a coarsely roughened surface. ca. X 2,000.

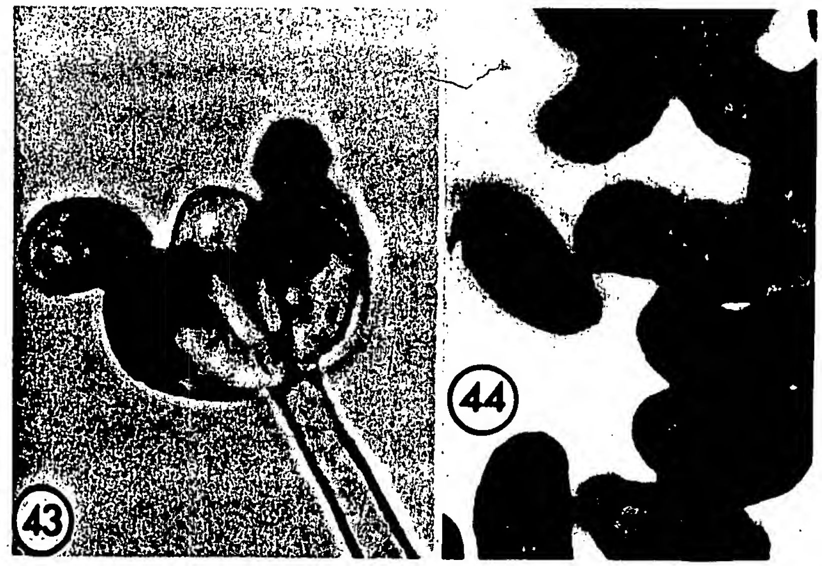
recognize it as a species and make a new combination *Stachybotrys microspora* (Mathur & Sankhla) Jong & Davis.

The strain ATCC 22218 originally designated as *S. atra* var. *microspora* at the Commonwealth Mycological Institute as IMI 136,344 appears to be a typical *S. chartarum* and is now disposed under the latter name at the ATCC.

The temperature range for growth and conidial germination of ATCC 18852 is from 15 to 37 C. No clearing on cellulose agar medium by growth reveals that the fungus is unable to utilize the cellulose as a main carbon source.



Figures 41-42. *Stachybotrys nephrospora* ATCC 18839. 41. Phialides. ca. X 3,000. 42. Phialoconidia. ca. X 3,000.



Figures 43-44. *Stachybotrys nephrospora* ATCC 18839. 43. Phialides showing wide terminal openings through which phialoconidia are being produced. ca. X 3,000. 44. Comma-shaped phialoconidia featuring a coarsely roughened surface. ca. X 3,000.

Stachybotrys nephrospora Hansford, Proc. Linn. Soc. London 155: 45. 1943.

= *Stachybotrys reniformis* Tubaki, Trans. Mycol. Soc. Japan 4: 86. 1963.

= *Stachybotrys sinuatophora* Matsushima, Bull. Nat. Sci. Mus. Tokyo 14: 476. 1971.

Figures 41-44.

Growth on CMA somewhat restricted, attaining a diameter of 4-5 cm in 3 weeks, appressed, uniformly dense, uncolored at first, becoming dark granulate as conidial production commences. Margin of colony distinct due to

the absence of conidia, white, with compact hyphae. No staining of medium in advance of mycelium. Reverse olive green. Conidial production abundant a week following inoculation, first in the center, then centrifugally toward the margin of colonies, becoming zonate.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple, occasionally branched, 2-4 septate, at first hyaline, becoming olivaceous at the upper parts, up to 400 μm long, 3-5 μm wide, sometimes minutely rough-walled at the upper parts or throughout the length, sometimes more or less smooth throughout, slightly enlarged at the apex which bears terminal phialides in a whorl of 4-9 around a central phialide.

Phialides enteroblastic, determinate, discrete, unicellular, olivaceous, obovate or ellipsoid, smooth-walled, 10-12 X 5-6 μm, with conspicuous collarettes.

Phialoconidia acrogenous, arising singly and successively as separate units from an open growing point at the apex of the phialide, bent away outwardly from the axis even horizontally sliming down at once to form a mucilaginous black mass which envelopes the tips of the phialides, at first hyaline and smooth-walled, when mature, dark olive, more or less opaque, coarsely roughened, unicellular, reniform or comma-shaped, 10-12 X 4-5 μm.

REMARKS: *Stachybotrys nephrospora* was first discovered by Hansford (1943) on dead wood in Kamplala, Uganda. The type specimen (Hansford 1114) was deposited in the Kew Herbarium (K), Royal Botanic Garden, England. It is readily recognized by the reniform phialoconidia which develop obliquely or even horizontally from the wide open apex of the phialide. Tubaki (1963) described a new species, *Stachybotrys reniformis*, from the decayed leaves of an herb (Labiatae) in Ambo, Yaki Island, Japan. Since the shape and size of phialoconidia agree fairly well with those of *S. nephrospora*, Verona and Mazzucchetti (1968) suggested that *S. reniformis* and *S. nephrospora* were possibly synonymous.

Matsushima (1971a,b) recently isolated a similar fungus from Goroka soil, Papua-New Guinea; however, he described it as a new species, *Stachybotrys sinuatophora* Matsushima. This isolate (MFC-2690) was deposited in the ATCC and given accession number ATCC 22706.

The description presented is based upon the type culture (ATCC 18839 = IFO 7067) of *S. reniformis* which was obtained from Institute for Fermentation (IFO) in Osaka, Japan. No culture is available for *S. nephrospora* which is known from the type collection only. Comparative studies of the type specimen of *S. nephrospora* and the type cultures of both *S. reniformis* and *S. sinuatophora* have led to the conclusion that they are identical. It is therefore proposed that *S. reniformis* Tubaki, 1963, and *S. sinuatophora* Matsushima, 1971, be regarded as later, facultative synonyms of *S. nephrospora* Hansford, 1943.

Both ATCC 18839 and ATCC 22706 grow and sporulate well on cornmeal agar at temperatures ranging from 15 to 30 C. However, ATCC 18839 is capable of producing cellulase on the cellulose agar medium and ATCC 22706 is unable to do so.

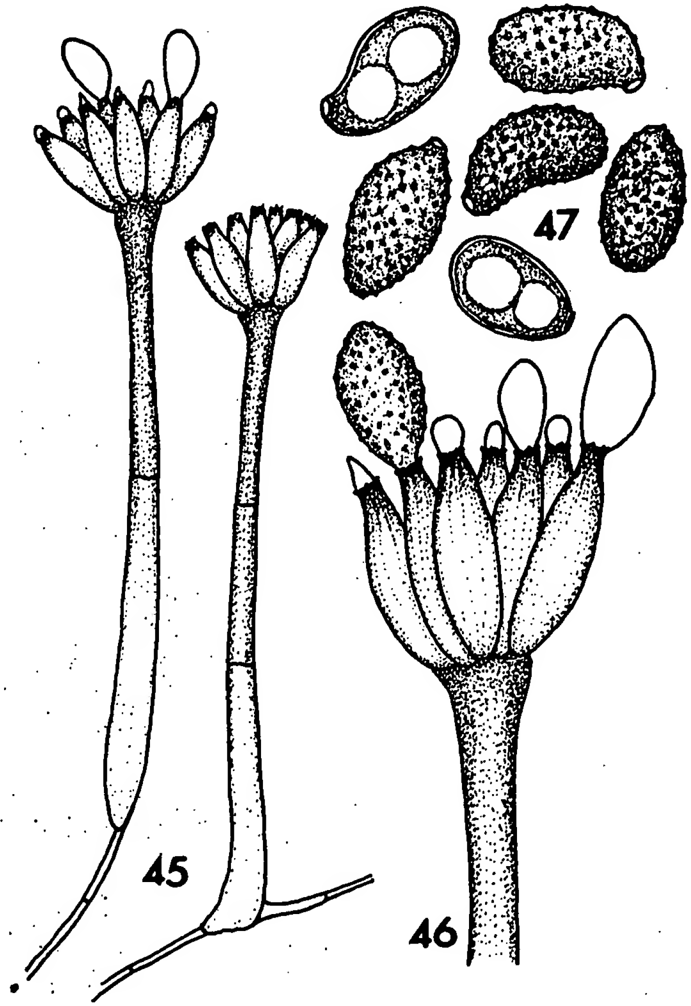
Stachybotrys nephrospora is characterized by its synpodially branched conidiophores and its reniform phialoconidia.

Stachybotrys oenanthes M. B. Ellis, CMI Mycol. Papers 125: 29. 1971.

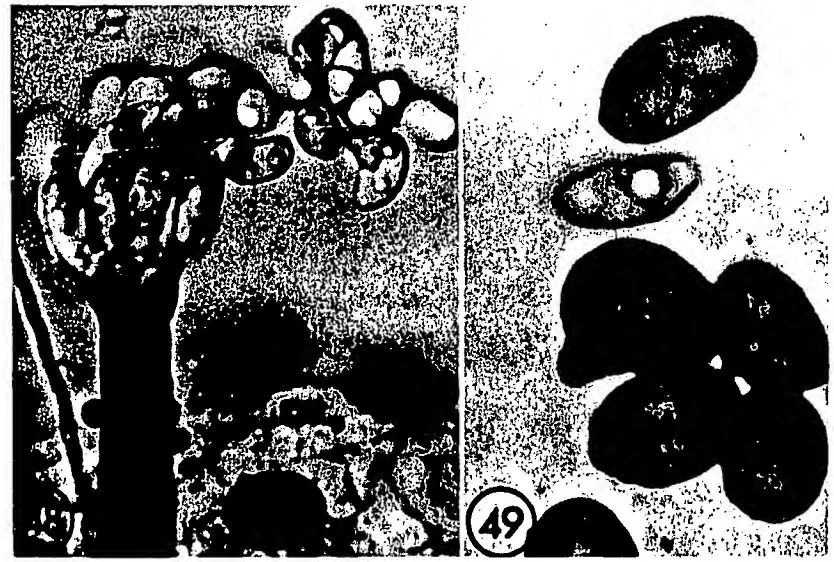
Figures 45-49.

Growth on CMA somewhat restricted, reaching 3 cm in diameter in 2 weeks. The surface of colony downy, orange at first, becoming dark granulate at the central part of the colony as conidia develop. Reverse stained bright orange. Conidia produced abundant a week after inoculation of plates.

Conidiophores determinate, macronematous, erect, straight or slightly curved, simple, 1-2 septate, smoke gray to black, smooth-walled, up to 190 μm long, 5-7 μm wide, slightly enlarged at the apex which bears terminal phialides in a whorl of 8-20 around a central phialides.



Figures 45-47. *Stachybotrys oenanthes* ATCC 22844.
45. Conidiophores with phialides and phialoconidia. ca. X 1,000. 46. Phialides and phialoconidia. ca. X 2,000.
47. Mature phialoconidia. ca. X 2,000.

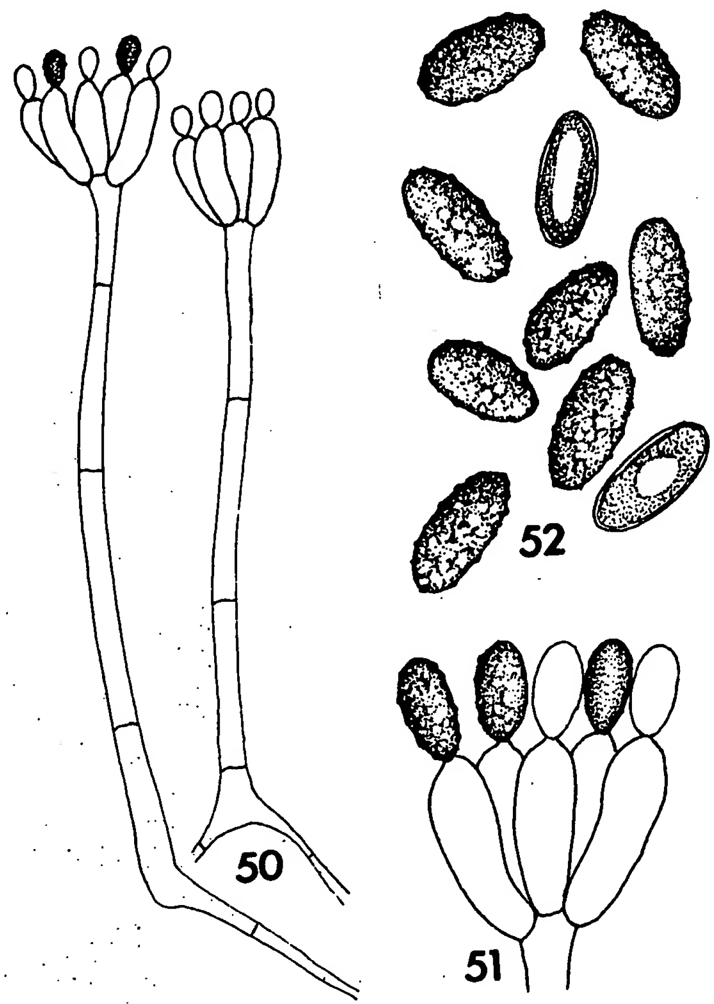


Figures 48-49. *Stachybotrys oenanthes* ATCC 22844.
48. Phialides and immature phialoconidia. ca. X 1,500.
49. Mature phialoconidia. ca. X 2,000.

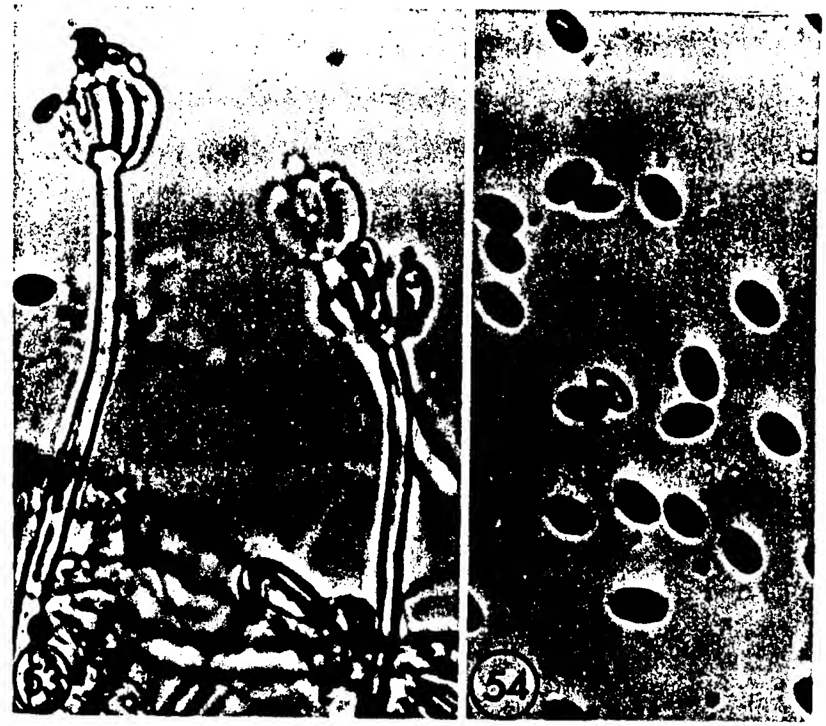
Phialides enteroblastic, determinate, discrete, unicellular, cylindrical, smoke gray to black, smooth-walled, 10-20 X 5-7 µm, with conspicuous collarettes.

Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline and smooth-walled, when mature smoke gray to black, heavily verrucose, ovoid to reniform, sometimes obliquely attenuate at the base, 9-10 X 5-7 µm.

REMARKS: The strain ATCC 22844 (=IMI 16,185) studied is the type culture isolated by M. B. Ellis (1971) from dead stems of *Oenanthe crocatae* in Quernsey. It grows and sporulates on CMA at temperatures in the range of 10 to 24 C. The upper limit of temperature for spore germination is 30 C. No production of clearing on the cellulose agar medium by growth indicates that it is not a cellulose-decomposing fungus.



Figures 50-52. *Stachybotrys parvispora* ATCC 18877. 50. Conidiophores with phialides and phialoconidia. ca. X 1,000. 51. Phialides and phialoconidia. ca. X 3,000. 52. Mature phialoconidia. ca. X 3,000.



Figures 53-54. *Stachybotrys parvispora* ATCC 18877. 53. Growth habit of conidiophores. ca. X 1,000. 54. Phialoconidia. ca. X 1,000.

Stachybotrys parvispora Hughes, CMI Mycol. Papers 48: 74. 1952.

Figures 50-54.

Growth on cornmeal agar somewhat restricted, reaching 4 cm in diameter in 3 weeks. Colonies felty to silky, white at first, becoming olive green in areas of copious conidial production. Margin of colony distinct, white, with compact hyphae. Reverse stained light orange. Conidia produced in abundance a week after inoculation of plates.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple, occasionally branched, 3-5 septate, hyaline, smooth-walled, up to 200 µm long, 2-5 µm wide, the basal cell slightly inflated, attenuated toward the tip, slightly enlarged at the apex which bears terminal phialides in a whorl of 4-10 around a central phialide.

Phialides enteroblastic, determinate, discrete, clustered in a verticillate arrangement, unicellular, subclavate, hyaline, smooth-walled, 8-11 X 3-4 µm, with conspicuous collarettes.

Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline, when mature, dark olive gray, more or less opaque, coarsely roughened, unicellular, ovate, 5-6 X 3-3.5 µm.

<u>REMARKS</u>: *Stachybotrys parvispora* was originally described by Hughes (1952) from dead leaves of *Ananas*, *Ficus* and *Setariae* from the Gold Coast of tropical Africa. It is readily recognized by the small dark olive phialoconidia 3 to 6 µm long by 3 to 3.5 µm wide. The strains ATCC 18877 (=IMI 62,388) and ATCC 18876 (=IMI 106,334) studied were secured from the Commonwealth Mycological Institute; the former was isolated from soil in Congo and the latter from root of *Hevea* in Malaya. Both fit Hughes' original description.

The growth temperature range for ATCC 18877 is narrow, 20 to 30 C. However, the upper limit of temperature for conidial germination is 37 C. No clearing of the cellulose agar medium by growth of this fungus indicates that it is unable to decompose cellulose.

Stachybotrys theobromae Hansf., Proc. Linn. Soc. Lond. <u>155</u>: 45. 1943.

Figures 55-60.

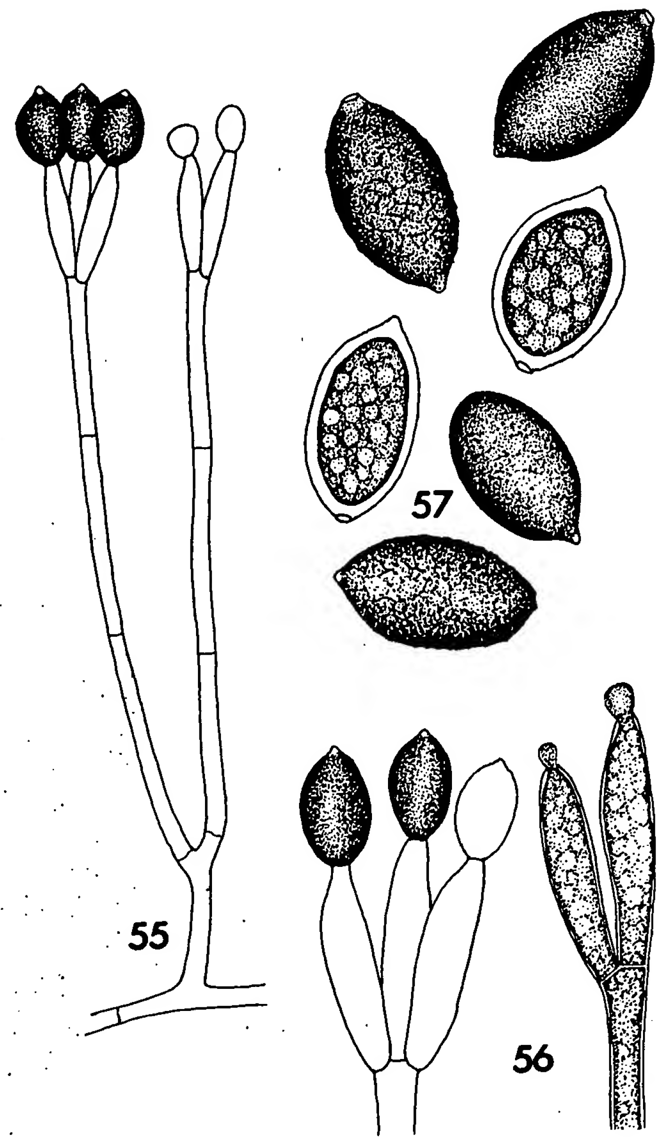
Growth on potato dextrose agar covering the Petri-dish plates in 3 weeks, fleecy to velvet lanose with various degrees of luxuriance, gleaming white, becoming dark granulate as conidial production commences. Margin of colony distinct, lobed, peripheral hyphae dispersed. No staining of medium in advance of mycelium. Reverse at first uncolored, later becoming olive. Conidia produced in abundance two weeks after inoculation of plate.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple or irregularly branched, 6-14 septate, hyaline, length variable, up to 400 µm long, 3-5 µm wide, the basal cell slightly inflated, attenuate toward the tip, smooth throughout the length, slightly enlarged at the apex which bears a terminal phialide or in a group of 3-4 around a central phialide.

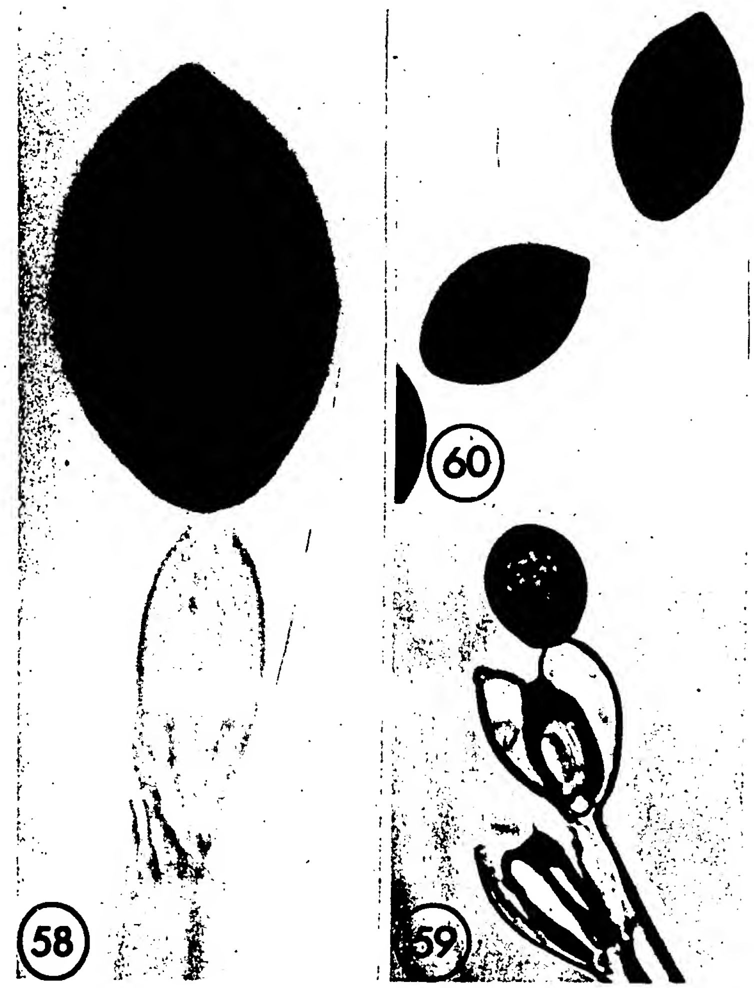
Phialides enteroblastic, determinate, discrete, unicellular, subclavate, hyaline, smooth-walled, 15-23 X 4-5 µm, with conspicuous collarettes.

Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline and smooth-walled, when mature, dark green, more or less opaque, coarsely roughened, unicellular, oval, 16-28 X 12-16 µm, usually with a well-marked apical apiculus.

<u>REMARKS</u>: The fungus was first discovered by Hansford (1943) on branches of *Theobroma cacao* L. in Uganda. The phialoconidia are described as 15-18 X 20-28 µm on phialides 7-10 X 12-28 µm. Hughes (1952) reported several collections from the Gold Coast on twigs of *Bura crepitans* L. and on branches and dead dry empty pods of *Theobroma cacao*. Hughes described and illustrated the phialoconidia with a well-marked basal apiculus. Hughes' figures were reproduced by Verona and Mazucchetti (1968). However, careful examinations of strain ATCC 18905 (=IMI 105,321) which was originally isolated by T. H. Williams from *Theobroma cacao* in Tuaran. Sabah, Malaysia, and the type specimen deposited with the Kew Herbarium (K) indicate that the apiculus is apical instead of basal. This species differs from other *Stachybotrys* species studied by having large, dark green phialoconidia on hyaline phialides and conidiophores.



Figures 55-57. *Stachybotrys theobromae* ATCC 18905. 55. Conidiophores with terminal phialides and phialoconidia. ca. X 500. 56. Phialides and phialoconidia. ca. X 800. 57. Phialoconidia with a well-marked apical apiculus. ca. X 1,200.



Figures 58-60. *Stachybotrys theobromae* ATCC 18905. 58. Phialide bearing a terminal phialoconidium with a well-marked apical apiculus. ca. X 2,300. 59. Phialides. ca. X 800. 60. Mature phialoconidia. ca. X 1,000.

Stachybotrys crassa El. Marchal (1895) and *S. nilagirica* Subramanian (1957) are also described as having large globose phialoconidia, 16-18 µm in the former and 15.4-28 µm in the latter. Unfortunately, neither have been cultured. However, the original descriptions and figures of both species show that they are quite distinct from *S. theobromae* in which the phialoconidia are oval and with a well-marked apical apiculus.

Stachybotrys crassa has been reported only from the type collection on dung in Belgium. Bisby (1943) in his review of the genus considered it a doubtful species unless the type specimen can be examined, or the species rediscovered.

ATCC 18905 grows and sporulates well on potato dextrose agar at temperatures in the range of 20 to 30 C. However, the maximum temperature for conidial germination is 37 C. The fungus is unable to clear the cellulose agar medium, indicating that it is not a cellulose-decomposing fungus.

Memnoniella echinata (Riv.) Galloway, Trans. Brit. Mycol. Soc. <u>18</u>: 165. 1933.

≡ *Penicillium echinatum* Riv., Dei Parassiti Vegetali, p. 451. 1873.

≡ *Haplographium echinatum* (Riv.) Sacc., Syll. Fung. <u>4</u>: 307. 1886.

≡ *Stachybotrys echinata* (Riv.) Smith, Trans. Brit. Mycol. Soc. <u>45</u>: 392. 1962.

= *Periconia papyrogena* Sacc., Michelia <u>1</u>: 273. 1878.

≡ *Stachybotrys papyrogena* (Sacc.) Sacc., Fungi ital. Tab. 900. 1881.

≡ *Sterigmatobotrys papyrogena* (Sacc.) Oud., Nederl. Kruidk. Arch., II, <u>4</u>: 548. 1886.

= *Memnoniella aterrima* Höhnel, Zbl. Bakt. (Abt. II) <u>60</u>: 16. 1923.

Figures 61-66.

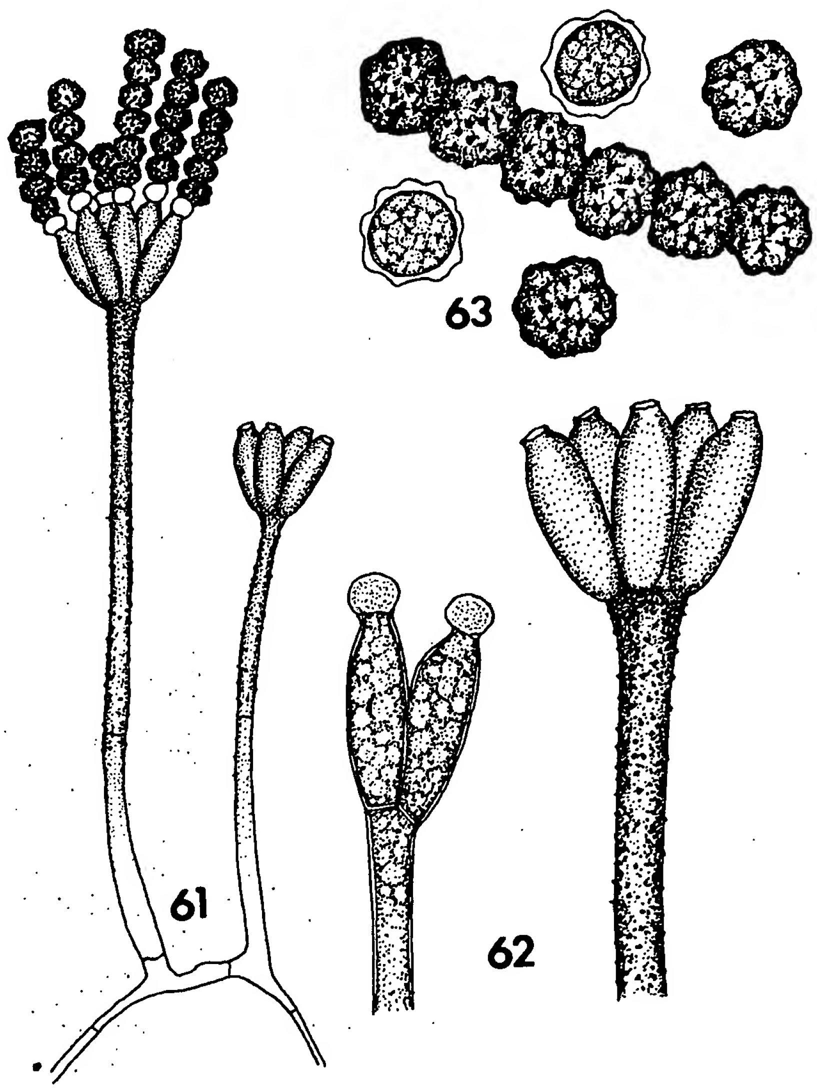
Growth on cornmeal agar somewhat restricted, reaching 5 cm in diameter in 3 weeks, at first submerged and colorless, becoming downy and dark granulate as conidial production commences. Margin of colony not distinct, with submerged hyphae and few conidia. Reverse stained yellowish brown to brownish gray. Conidia produced in abundance 2 days after inoculation on agar plates and sterilized rabbit dung.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, unbranched, 1-3 septate, at first hyaline, later olivaceous, 70-90 µm long, 3-5 µm wide, the basal cell slightly inflated, sometimes minutely rough-walled throughout the length, sometimes more or less smooth throughout, slightly enlarged at the apex which bears terminal phialides in a whorl of 6-9 around a central phialide.

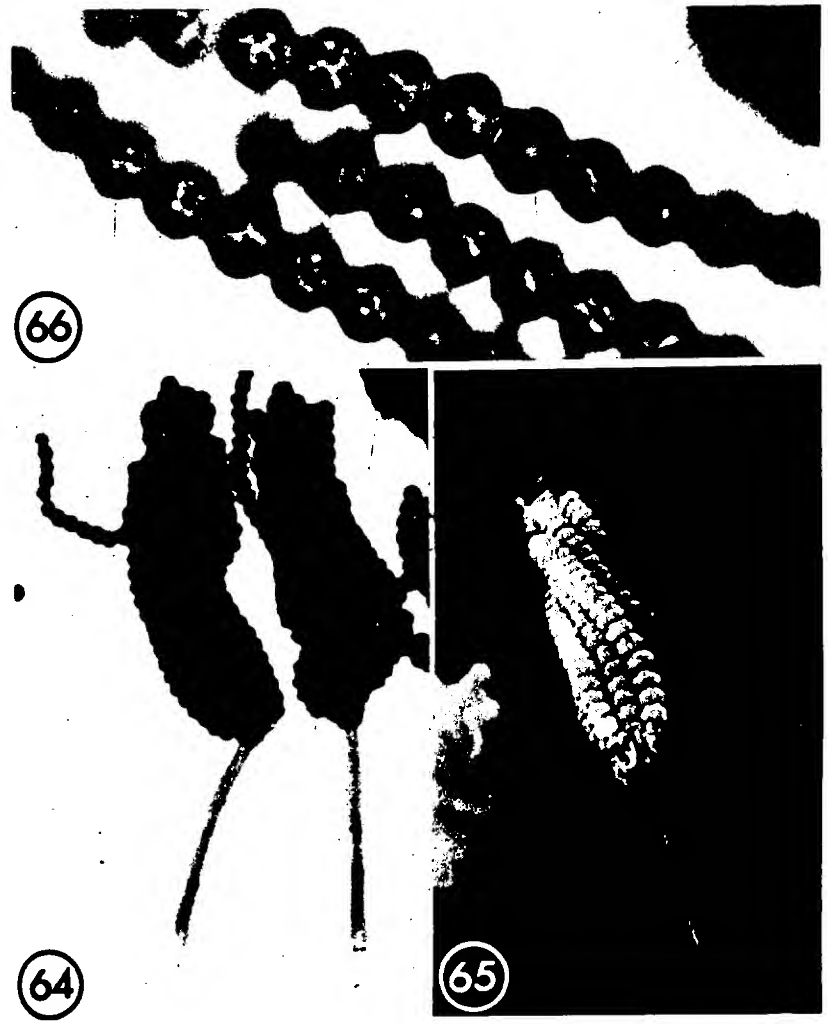
Phialides enteroblastic, determinate, discrete, unicellular, pale olivaceous, obovate or ellipsoid, smooth-walled, 7-10 X 3-4 µm with conspicuous wide terminal collarettes.

Phialoconidia acrogenous, arising in basipetal succession and remaining attached to one another by a common septum in long persistant chains, at first hyaline and smooth-walled, when mature, dark olive gray, more or less opaque, coarsely warted, continuous, octagonal-globose in side view and circular in outline in end view, dry (not in slime), 3-6 µm in diameter.

<u>REMARKS</u>: Rivolta (1873) first discovered this fungus on hay and wheat culms in Northern Italy and described it as *Penicillium echinatum* Rivolta (not *P. echinatum* Dale). The description and figure given by Rivolta indicated that the distinguishing feature of this fungus was the black catenulate phialoconidia resembling those of *Aspergillus niger* and the conidiophores with penicillate heads resembling those of *Stachybotrys*. However, Saccardo (1186) later transferred Rivolta's species from *Penicillium* Link to *Haplographium* Berk. & Br. which is characterized by slimy masses of blastoconidia borne singly at the apex of dark conidiophores with penicillate heads (Barron, 1968).



Figures 61-63. *Memmoniella echinata* ATCC 11974.
61. Conidiophores with terminal phialides and phialoconidia in chains. ca. X 600. 62. Phialides. ca. X 1,200.
63. Phialoconidia. ca. X 2,400.



Figures 64-66. *Memnoniella echinata* ATCC 11974.
64. Growth habit of conidiophores. ca. X 200.
65. Scanning electron micrograph of phialides with phialoconidia in chains. ca. X 600. 66. Mature phialoconidia in chains. Note a coarsely warted surface. ca. X 2,000.

Unaware of Rivolta's work, Höhnel (1923) described this species based on his isolate from cotton yarn in Vienna and designated it as *Memnoniella aterrima* gen. and sp. nov. Galloway (1933) critically reviewed Rivolta's and Höhnel's works and accepted Höhnel's genus, making the combination *M. echinata* (Riv.) Galloway for his isolate obtained as an air contaminant in the laboratory. He also pointed out that this fungus is closely related to *Stachybotrys* from which it is distinguished by the globose phialoconidia borne in chains. Further examinations of the type material of *M. aterrima* in the Farlow herbarium by Linder (Galloway, 1933) and by White *et al.* (1949) have confirmed Galloway's decision that *P. echinatum* and *M. aterrima* belong to the same species.

Upon reexamination of Galloway's original culture of *M. echinata*, Bisby (1943) suggested the possibility that *M. echinata* might be an unusual or abnormal form of *Stachybotrys subsimplex* in which slime production was reduced to allow retention of the conidia in chains. However, Bisby (1945) later recognized *M. echinata* as being distinctive from *S. subsimplex* after studies of several new cultures from different localities. Although Zuck (1949) found that some isolates of *Memnoniella* occasionally producing a *Stachybotrys*-like phase much like the description of *S. subsimplex sensu* Bisby (1943), he considered *M. echinata* a distinct species since some of his cultures had remained stable for eight years in the characters Galloway listed for *M. echinata*. Padwick (1945) accepted *M. echinata* and redescribed it based on his isolate from cotton cardage in Cawnpore, India. Padwick's description was recently reproduced by Verona and Mazzucchetti (1968).

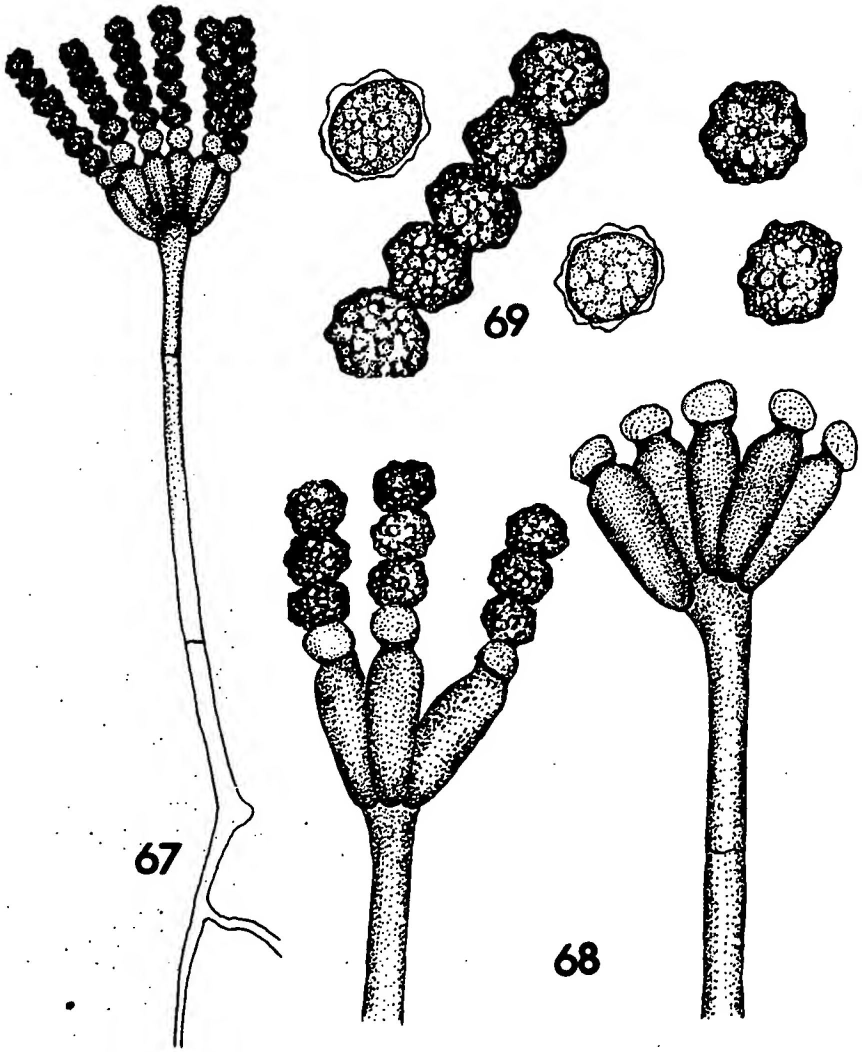
The species concept of *S. subsimplex* has recently been clarified by Deighton (1960) who points out that the type material (IMI 10,941) has globose catenulate phialoconidia resembling those of *M. echinata* from which it differs in its larger phialoconidia. It is obviously not a *Stachybotrys*-like phase of *M. echinata* but a distinct species of *Memnoniella*. Deighton therefore made the new combination *Memnoniella subsimplex* (Cooke) Deighton. Nonetheless, Smith (1962) would relegate *Memnoniella* to synonymy with *Stachybotrys*, making a new combination *Stachybotrys echinata* (Riv.) Smith for *M. echinata* (Riv.) Galloway. Because the culture studied in the present work also appears to remain stable in the characters defining *Memnoniella*, we recognize *Memnoniella* and *Stachybotrys* as distinct genera but very closely related.

The nomenclatural synonyms of *Memnoniella echinata* listed above are compiled from the opinion of Smith (1962).

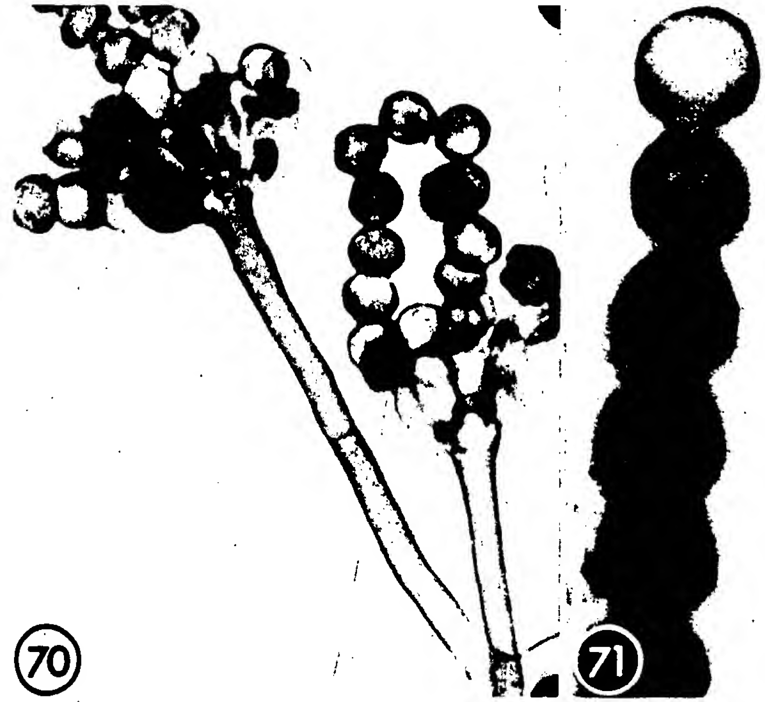
An excellent report on the history, distribution and economic significance of *M. echinata* has been published by White, Yeager and Shotts (1949). From an analysis of samples of deteriorated fabrics received from numerous military installations around the tropical belt of the world and from the United States, and after examinations of isolates from such samples, and of herbarium specimens, they concluded that *M. echinata* is a tropical cellulose-loving species that is able to utilize cellulose as a major carbon source.

The strain ATCC 11974 studied was obtained from E. G. Simmons as QM 1225 of the U. S. Army Natick Laboratories, Natick, Massachusetts. This strain was also included by McQuade (1963) in his studies of morphogenetic responses of fungi to factorily arranged variation in concentrations of NH_4Cl, K_2HPO_4 and glucose. It grows and sporulates well on cornmeal agar at temperatures in the range of 15 to 30 C. The upper limit of temperature for conidial germination is 37 C. Production of clearing by growth on the cellulose agar medium reveals that it is a strong cellulose-decomposing fungus. ATCC 11974 is being used for fungus resistance tests in U. S. military specifications. The other two strains (ATCC 22697 and ATCC 22698) studied were isolated by T. Matsushima from forest soil in Papua-New Guinea.

Two additional species of *Memnoniella* have been reported from India. *Memnoniella levispora* Subramanian (1954) has conidia with smooth walls, 3-7 µm in diameter and also been recovered from Pakistan (Ellis, 1971). *Memnoniella zingiberis* Rao has conidia with roughened walls, 4.4-6.8 µm in diameter. According to the description and figure given by Rao (1962), it is quite possible that *M. zingiberis* is a synonym of *M. echinata*. Unfortunately, neither *M. levispora* nor *M. zingiberis* is available in culture.



Figures 67-69. *Memmoniella subsimplex* ATCC 18838.
67. Conidiophore with terminal phialides and phialoconidia in chains. ca. X 500. 68. Phialides. ca. X 1,000.
69. Mature phialoconidia. ca. X 1,600.



Figures 70-71. *Memmoniella subsimplex* ATCC 18838.
70. Phialides with phialoconidia in chains. ca. X 800.
71. Mature phialoconidia in chains. Note a coarsely warted surface. ca. X 2,000.

Memmoniella subsimplex (Cooke) Deighton, CMI Mycol. Papers 78: 5. 1960.

≡ *Stachybotrys subsimplex* Cooke, Grevillea 12: 33. 1883.

= *Haplographium musae* Sawada, Nat'l. Taiwan Univ. Coll. Agric. Spec. Publ. 8: 193. 1959.

Figures 67-71.

Growth on cornmeal agar somewhat restricted, reaching 4 cm in diameter in 3 weeks, submerged, colorless to

yellowish pink. Central parts of old colonies becoming downy and dark granulate as conidial production commences. Margin of colony not distinct, hyaline, with submerged hyphae. Reverse stained orange to brown. Conidia produced in abundance a week after inoculation of the plate.

Conidiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, unbranched, 1-3 septate at first hyaline, later olivaceous, up to 220 µm long, 3-4 µm wide, the basal cell slightly inflated, sometimes minutely rough-walled throughout the length, sometimes more or less smooth throughout, slightly enlarged at the apex which bears terminal phialides in a whorl of 5-12 around a central phialide.

Phialides enteroblastic, determinate, discrete, unicellular, ellipsoid, olivaceous, smooth-walled, 12-15 X 5-6 µm, with conspicuous collarettes.

Phialoconidia acrogenous, arising in basipetal succession and remaining attached to one another by a common septum in long persistent chains, at first hyaline and smooth-walled, when mature, dark olive gray, more or less opaque, coarsely warted, continuous, octagonal-globose in side view and circular in outline from the end view, dry (not in slime), 6-9 µm in diameter.

REMARKS: In reviewing the genus *Stachybotrys*, Bisby (1943) tentatively emended *S. subsimplex* Cooke to include *Memnoniella echinata* (Riv.) Galloway. Zuck (1946) later amplified Bisby's suggestion, indicating that *M. echinata* in culture may sometimes produce a *Stachybotrys*-like phase similar to the description of *S. subsimplex sensu* Bisby. As pointed out by Bisby and Ellis (1949), this leaves the name *Stachybotrys* as doubtful, unless it refers to the *Stachybotrys*-like phase of *Memnoniella echinata*.

However, the type material of *S. subsimplex* in the Kew Herbarium (K) was reexamined by Deighton (1960) who found that it is not a *Stachybotrys*-like phase of *M. echinata* but a distinct species of *Memnoniella* . He therefore transferred Cooke's species from *Stachybotrys* to *Memnoniella* as *M. subsimplex* (Cooke) Deighton. Phialoconidia of this fungus differ from those of *Stachybotrys chartarum* (as *S. atra*) and other *Stachybotrys* species in being slightly flattened along the axis from the base

to the apex instead of being elongated along this axis, and in not sliming down at once into a mucilaginous mass, but instead, remaining for a period attached in long chains. Deighton also pointed out that it is obviously closely related to *M. echinata* from which it differs in its larger phialoconidia, (6)7-8(9) µm in diameter, as against 4-5.5(6) µm in *M. echinata*.

The fungus commonly occurs on dead *Musa* leaves and on dead parts of other host plants in the tropics (Deighton, 1960; Sawada, 1959). It was also recorded by Joffe (1967) from soil in a citrus fertilizer trial in Israel and by Matsushima (1971a) from forest soil in the Solomon Islands.

The strain ATCC 18838 studied was obtained from K. Tubaki of the Institute for Fermentation, Osaka, Japan, as *Stachybotrys echinata* (Riv.) Smith (IFO 7525). ATCC 22699 and ATCC 22700 both were isolated by T. Matsushima from forest soil in British Solomon Islands. Morphologically, they fit with Deighton's description of *M. subsimplex* and are readily distinguishable by the predominance of catenulate phialoconidia, and their size. During the course of the present work, we have not seen a *Stachybotrys*-like phase in these cultures. The growth temperature range of ATCC 18838 is very narrow, 24 to 30 C. The upper limit of temperature for conidial germination is also 30 C. The fungus is able to decompose cellulose and utilize it as a sole carbon source.

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